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PROCEDURAL GUIDELINES FOR ECOLOGICAL RISK ASSESSMENTS AT U.S. ARMY SITES VOLUME II - RESEARCH AND BIOMONITORING METHODS FOR THE CHARACTERIZATION OF ECOLOGICAL EFFECTS

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The purpose of this report is to provide guidance for procedures to conduct ecological risk assessment (ERA) for use by risk assessors under contract to the U.S. Army Environmental Center (AEC) at Army National Priority (NPL) sites and sites listed under the Base Realignment and Closure (BRAC) program. This report contains information on more than 100 environmental models and test methods. These methodologies can assist the risk assessor in the RI/FS process to select appropriate methods to address the issues of concern.

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PREFACE

The work described in this report was authorized under MIPR No. 2372 from the U.S. Army Environmental Center (AEC) and Work Order No. 56015408-05-0000 from the U.S. Army Edgewood Research, Development and Engineering Center (ERDEC).* This work was started in September 1992 and completed in December 1994.

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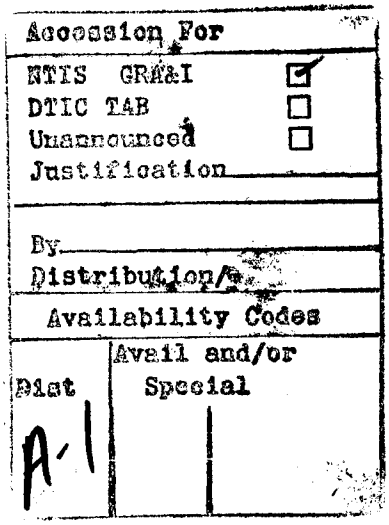

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PROCEDURAL GUIDELINES FOR ECOLOGICAL RISK ASSESSMENTS AT U.S. ARMY SITES VOLUME II

Research and Biomonitoring Methods for the Characterization of Ecological Effects

INTRODUCTION

Volume 2 of this report contains summaries of research and biomonitoring methods useful in characterizing ecological effects at hazardous waste sites. Appendix A of this volume contains summaries of models useful for contaminant distribution, fate and biological uptake. Appendix B contains biological test methods that can be used to characterize exposure or effects in biota. Appendix C contains biological test methods presently under development, but expected to be of future use in ecological risk assessment. The models and techniques presented in Appendices A and B are intended to present the project manager or risk assessor (RA) with an overview of test methods. Further, this volume is intended to provide references to which assessment personnel can refer to learn more about the appropriateness or suitability of a particular test. The summaries are meant as an "opening" to the techniques and literature. The RA should not rely on them to be the sole source of information when deciding which tests to use to characterize ecological effects.

It is important for the RA to realize that there may be considerable variation in certain of the described test methods, as many of the summaries describe closely related tests. Summaries of this sort describe tests in which different analytical techniques may be used to assess the same or similar test endpoints. Also, many of the biochemical/physiological measures are under continual development. New ideas and approaches to ecological assessment require ongoing methods development. Hence, if a test is deemed to be of potential interest, it behooves the RA to contact experts in the field to get the most current developments in test methods.

In Appendix B, there may be considerable variation from the stated times required to perform a particular test. During the development of these summaries, the amount of training or time required to perform a particular test was often difficult to categorize. For example, many biochemical tests require very little time to run individual samples; however, it is seldom the case when characterizing ecological effects that the RA or contract personnel perform analyses on single samples. As is the case with field testing, assessment of many samples may be arduous and time consuming.

The Technical Summaries are organized in a manner intended to make the information easy to assimilate. The categories for each

summary (e.g., description, references) are self explanatory; however, a few need further explanation. The category "Logistical Considerations" is subdivided into two subfields, "Sample Collection" and "Sample Analysis." Categorical entries of "Minimal," "Moderate," and "Extensive" have been used instead of definitive values for these subfields. We have defined the categories as follows:

Training

Extensive - six months experience or greater performing the analysis or sample collection.

Moderate - Less than six months experience but more than a high school education.

Minimal - High school education.

Time

Intensive - three months or more to assess a single sample.

Moderate - one week to three months.

Minimal - Less than one week.

APPENDIX A

MODELS OF USE IN ECOLOGICAL RISK ASSESSMENT

Model Name: AERIS
Model Type: Fate, Multimedia

Description:

This is a risk assessment model that estimates environmental concentrations and subsequently, human exposure in the vicinity of contaminated land sites. It is intended for use at sites where redevelopment is under consideration. The model runs within a user-friendly expert system programming environment. An "intelligent" preprocessor interrogates the user about the redevelopment scenario to be assessed, assisting where necessary, or supplying default values.
It estimates pseudo steady-state concentrations of contaminant in compartments such as air and groundwater based on the concentration of the contaminant in the soil. These predicted environmental concentrations are used to estimate the exposure incurred by a site user. It is intended to provide a consistent approach to establishing soil guidelines and identifying cleanup objectives.

Key References:

Senes Consultants. 1989. Contaminated Soil Cleanup in Canada, vol. 5, Development of the AERIS model, Final Report prepared for the Decommissioning Steering Committee.

Senes Consultants. 1989. Contaminated Soil Cleanup in Canada, vol. 6, User's guide for the AERIS model, Prepared for the Decommissioning Steering Committee.

Logistical Considerations:

Equipment: IBM-PC compatible computer

Critique/Comments:
Use of the model requires many input parameters. The model employs a user friendly interactive computer program to examine on-site human health risks of relatively old contamination. It is inappropriate for recent spills. AERIS allows the user to calculate risks from a site or to develop cleanup levels. It lacks transport features and is not useful for predicting the fate of complex mixtures. Unfortunately, the model is currently unavailable for public use.

Model Name: Aquatic Food Chain Models
Model Type: Exposure, Bioaccumulation, Aquatic, Toxicant Uptake, Food Chain

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Description:

The models were developed for calculating the concentration of organic chemicals in a simple generic aquatic food chain. Chemical uptake efficiency from water, excretion rate and chemical assimilation efficiency are variable as a function of the octanol water partition coefficient, K_{ow}. The models indicate the significance of the growth rate and variable efficiency of uptake in the calculation of a bioconcentration factor BCF under field conditions.
The models extend a previously developed steady state bioconcentration model of the distribution of chemicals as a function of trophic level in the ecosystem.

Key References:

Clark, J.R., F.A.P.C. Gobas and D. Mackay. 1990. Model of organic chemical uptake and clearance by fish from food and water. Environ. Sci. Technol. 24:1203-1213.

Landrum, P.E., H. Lee II and M.J. Lydy. Toxicokinetics in aquatic systems: model comparisons and use in hazard assessment. Environ. Toxicol. Chem. 11:1709-1725.

Thomann, R.V. 1988. Deterministic and statistical models of chemical fate in aquatic systems. In: Ecotoxicology: Problems and Approaches. Springer Advanced Texts in Life Sciences. Springer, New York. Chapter 10, pp. 245-277.

Thomann, R.V. 1989. Bioaccumulation model of organic chemical distribution in aquatic food chains. Environ. Sci. Technol. 23:699-707.

Thomann, R.V. and J.P. Connolly. 1984. Model of PCB in the Lake Michigan lake trout food chain. Environ. Sci. Technol. 18:65-71.

Logistical Considerations:

Equipment: IBM-PC compatible computer

Critique/Comments: The model equations have been used successfully to calculate the concentration of organic chemicals in a diversity of aquatic food chains.

Model Name: CREAMS (Chemicals, Runoff and Erosion from Agricultural Management Systems)
Model Type: Fate, Nutrient runoff, Inorganic Chemical

Description:

The CREAMS model is structured into three components: hydrology, erosion/sedimentation, and chemistry. The model is useful in simulating stormloads and sediment-associated and dissolved chemicals in the runoff, sedimentand percolate fractions. For example, it estimates soluble and sediment attached nitrogen (N) and phosphorus (P) in runoff. A nitrogen submodel also considers plant uptake, denitrification, mineralization of organic nitrogen and leaching of nitrate. Fertilizer nitrogen and phosphorus can be added to the surface or incorporated in the profile in single or multiple applications during the year.

Key References:

Heatwole, C.D., K.L. Campbell and A.B. Bottcher. 1988. Modified CREAMS Nutrient Model for Coastal Plain Watersheds. Trans. Amer. Soc. Ag. Engineers 31:154-160.

Knisel, W.G. 1980. CREAMS: A field scale model for chemical runoff, and erosion from agricultural management systems. U.S.D.A. Conservation Research Report Number 26.

Knisel, W.G., G.R. Foster and R.A. Leonard. 1983. CREAMS: A system for evaluating management practices. In: Schaller, F.W. and G.W. Bailey (eds.) Agricultural Management and Water Quality Iowa State University Press, Ames, pp. 178-199.

Logistical Considerations:

Equipment: Unavailable

Critique/Comments: A major utility of CREAMS has bee the evaluation of alternate management practices for control or minimization of runoff of sediment and chemicals. Several alternate practices might be proposed for a given site. Each could be evaluated with CREAMS, and the responsible party could select a practice to minimize chemical movement offsite.CREAMS has been tested in a number of research watersheds in several land resource areas. Results show that the model can applied successfully by estimating parameter values from information in Conservation Research No. 26 (Knisel, 1980). Use of observed data for a site may be useful in improving model accuracy for the site for Tier II or III testing, but is unnecessary for Tier I tests. Testing has shown that the model adequately represents changes in

management practices and that relative differences between practices are valid.

Model Name: Enpart (Environmental Partitioning Model)
Model Type: Fate, Exposure, Multimedia

Description:

Enpart was developed by the U.S. EPA as a first-level screening tool for new and existing organic chemicals of possible concern. It is a fugacity based model which estimates the steady-state equilibrium or dynamic partitioning of organic chemicals among environmnental compartments. It identifies dominant pathways and data gaps, and estimates the chemicals persistence and bioconcentration potential.

The data required by the model include the properties of the chemical and some environmental parameters such as soil and sediment density, suspended sediment and biota concentrations. The output is in the form of concentration ratios between compartments rather than absolute concentrations.

Key References:

OECD (Organization for Economic Cooperation and Development). 1989. Compendium of Environmental Exposure AssessmentMethods for Chemicals. Environment Monographs, No. 27, OECD, Paris.

Mackay, D. and S. Paterson. 1993. Mathematical models of transport and fate. In: G. Suter (ed.), Ecological Risk Assessment, Lewis Publishers, Chelsea, Michigan. pp. 129-152.

Logistical Considerations:

Equipment: Unavailable

Critique/Comments: It is an easy-to-use approximate method intended to indicate chemicals which may require further testing (Mackay and Paterson, 1993).

Model Name: EXAMSII
Model Type: Fate, Transport, Exposure, Aquatic

Description:

This is an interactive mass balance model developed at the U.S. EPA Research Laboratory in Athens, GA, which predicts the fate of organic contaminants in stratified surface waters as a result of continuous or intermittent releases. It is widely used by the EPA and other environmental agencies in the U.S.

The water body is subdivided into zones, or segments, the mass balance of each segment being described by a differential equation. The resulting set of equations, which describes the mass balance of the entire system, incorporates comprehensive transport and transformational processes. It allows for loadings by point or nonpoint sources, dry fallout or aerial drift, atmospheric wash-out and groundwater seepage to selected segments.

The user has the choice of three operating models determined by the complexity of the problem under study. The modes range from a steady-state solution for a continuous release of a contaminant to the dynamic solution of a time varying source. Input data requirements are generally intensive.

The latest verion of EXAMS is applied in formulating aquatic ecosystem models and rapidly evaluating the fate, transport and exposure concentrations of synthetic organic chemicals--pesticides, industrial materials and leachates from disposal sites. EXAMS contains an interactive Database Management System (DBMS) designed for storage and management of project databases. User interaction is enhanced with an on Command Line Interface (CLI), context sensitive help menus, an on-line data dictionary and CLI user's guide and plotting capabilities for review of output data. EXAMS has 20 output tables which both document datasets and provide integrated results for aid in ecological risk assessments.

Key References:

Burns, L.A., D.M. Cline and R.R. Lassiter. 1982. Exposure Analysis modeling system (EXAMS): User manual and system documentation. EPA/600/3-82/023, U.S. Environmental Protection Agency, Environmental Research Laboratory, Athens, GA.

Logistical Considerations:

Equipment: Vax

Critique/Comments: The software is available from the Center for Exposure Assessment Modeling (CEAM), Environmental Research Laboratory, U.S. EPA Athens, GA 30613. Their phone number is (706) 546-3130/ fax (706)-546-2018.

Model Name: FGETS (Food and Gill Exchange of Toxic Substances)
Model Type: Exposure, Bioaccumulation

Description:

This is a fortran simulation that predicts temporal dynamics of a fish's whole body concentration (μg chemical/ grams live weight fish) of non-ionic, non-metabolized, organic chemicals that are accumulated from water and food. The model is based on a set of diffusion and forced convection partial differential equations, coupled to a process-based fish growth formulation. The theoretical basis and equation development are presented in Barber, et al. (1991). The model also calculates the time to reach a lethal activity in fish assuming that the chemical has a narcotic mode of action.

The model considers both biological attributes of the fish and physico-chemical properties of the chemical that determine diffusive exchange across gill membranes and intestinal mucosa. Important biological characteristics used by the model include the fish's gill morphometry, body weight, and fractional aqueous, lipid and structural organic composition. Physico-chemical properties of importance include the chemical's aqueous diffusivity, molar volume, and n-octanol/water partition coeffecient (K_{ow}), which is used as a surrogate to quantify chemical patitioning into the fish's lipid and structural organic fractions. K_{ow} is used in calculating the fish's bioconcentration factor, molecular volume is used to estimate aqueous diffusivity, and melting point is used in conjunction with K_{ow} to calculate the chemical's activity within the fish.

The model is parameterized for a particular fish species by means of a morphological and physiological database that delineates the fish's gill morphometry, feeding and metabolic demands, and body composition. The database currently holds data for five fish families: salmonidae, centrarchidae, cyprinidae, percidae and ictaluridae.

Three simulation modes in FGETS v.3 are (a) laboratory, (2) food chain and (3) food web. The first mode is for description of bioconcentration or bioaccumulation under controlled laboratory conditions. The latter two modes are for modelling these processes in field conditions.

Key References:

Barber, M.C., L.A. Suàrez and R.R. Lassiter. 1991. Modelling bioaccumulation of organic pollutants in fish with an application to PCBs in Great Lakes salmonids. Canadian Journal of Fisheries and Aquatic Sciences 48:318-337.

Logistical Considerations:

Equipment: IBM-PC compatible computer

Critique/Comments: A maximum of 10 chemicals may be simulated simultaneously by the VAX version; the PC version has a limit of 4 chemicals. A maximum number of species is 5 for the VAX version; the PC version has a limit of 3 species. The maximum number of observations per species is 50 for the VAX version; the PC version has a limit of 20 observations per species. The maximum number of age classes per species is 15 for both VAX and PC versions. The software is available from the Center for Exposure Assessment Modeling (CEAM), Environmental Research Laboratory, U.S. EPA Athens, GA 30613. Their phone number is (706) 546-3130/ fax (706)-546-2018.

Model Name: GEOTOX
Model Type: Fate, Multimedia

Description:

A multimedia compartmental model developed under contract from the U.S. government (DOE and Army) which calculates chemical partitioning, degrading reactions, and diffusive and nondiffusive interphase transport. The concentrations estimated for environmental compartments are combined with human inhalation and ingestion rates and absorption factors to calculate exposure. It consists of eight compartments: air (gas), air (particles), biomass, upper soil, lower soil, groundwater, surface water and sediments. These compartmental media are assumed to be composed of subphases of gas, liquid and solid. Environmental dimensions and characteristics can be adjusted to represent other regions. Chemical partitioning between compartments, interphase transport, reaction and advective losses are described by first-order rate constants. The model can be applied to constant or time-varying chemical sources.

The soil is treated as three layers: upper soil layer, lower soil layer and groundwater zone. The soil layers are described by depth, bulk density, porosity, water content, and fraction organic carbon parameters. The groundwater zone consists of solids with fluid filled pore space. Processes of adsorption, ion exchange, precipitation, colloidal infiltration and irreversible mineralization in the groundwater are incorporated by means of sorption partitioning constants and expressions. The water phase consists of water, biota and suspended solids in equilibrium.

Concentration of a chemical in land biomass is calculated as the product of the soil concentration and the plant/soil partition coefficient. This is an equilibrium type expression incorporating vegetation production or growth rate. Animal biomass is not considered in the mass balance but is treated as an exposure vector.

The model output is in the form of environmental concentrations, intake by various exposure pathways and total intake. A measure of relative health risk can be calculated for a number of chemicals.

Key References:

McKone, T.E. and D.W. Layton. 1986. Screening the potential risks of toxic substances using a multimedia compartment model: Estimation of human exposure. Regul. Toxicol. Pharmacol. 6:359-380.

Logistical Considerations:

Equipment: Unavailable

Data Requirements:

- physical-chemical properties
- degradation rate constants
- emission data
- environmental characteristics
  for example, fraction of land surface covered by water and average depth

Critique/Comments:

Model Name: GLEAMS
Model Type: Fate, Groundwater Nutrient Loading

Description:

This is a modified version of the CREAMS model that takes into account vertical pesticide movement in the root zone. The model retains the same runoff component structure found in CREAMS, but links the hydrology, erosion and pesticide submodels into one program for better efficiency in computer operation. Input files for the model are simpler and the maximum simulation time is increased to 50 years.

Key References:

Leonard, R.A., W.G. Knisel and D.A. Still. 1987. GLEAMS: Groundwater loading effects of agricultural management systems. Trans. ASAE 30:1403-1418.

Knisel, W.G. and R.A. Leonard. 1986. Impact of irrigation on groundwater quality in humid areas. Water Forum 86. pp. 1508-1515. In: World Water Issues in Evolution. Proc. of ASAE Spec. Conf., Long Beach, CA. 4-6 Aug. Am. Soc. Civil Engineering, Boise, ID.

Logistical Considerations:

Equipment: Unavailable

Critique/Comments: The software is available from the Center for Exposure Assessment Modeling (CEAM), Environmental Research Laboratory, U.S. EPA Athens, GA 30613. Their phone number is (706) 546-3130/ fax (706)-546-2018.

Model Name: MINTEQAII
Model Type: Fate

Description:

This is an equilibrium metal speciation model applicable to metallic contaminants in surface and groundwaters. It is different in purpose than the mass balance models for organic contaminants. It calculates the equilibrium aqueous speciation, adsorption, gas phase partitioning, solid phase saturation states and precipitation-dissolution of 11 metals (arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, thallium and zinc). It contains an extensive thermodynamic base and is designed to make minimal demands on the user.

A degree of expertise regarding kinetic limitations at particular sites is required for proper application of the model. The output is a description of the major metal species in the system.

Key References:

Brown, D.S. and J.D. Allison. 1987. MINTEQAI Equilibrium metal speciation model: A user's manual. U.S. EPA, Athens, GA.

Logistical Considerations:

Equipment: IBM compatible PC

Critique/Comments: The software is available from the Center for Exposure Assessment Modeling (CEAM), Environmental Research Laboratory, U.S. EPA Athens, GA 30613. Their phone number is (706) 546-3130/ fax (706)-546-2018.

Model Name: Persistence
Model Type: Fate, Aquatic

Description:

This model was developed for the National Research Council of Canada as a screening method to estimate the fate of organic chemicals, especially pesticides, that are released into the aquatic environment. There are four compartments in the model: water, catch-all (including suspended solids, invertebrates and other aquatic life, excluding fish), sediment and fish. The model can calculate both steady-state or time-dependent solutions. Default environments for the model are a Standard Pond and a Standard Lake simulating a small eutrophic pond and a deep, oligotrophic lake. Removal pathways include photodegradation, volatilization and hydrolysis in water; biodegradation in fish; and microbial degradation in suspended solids and sediments.

Output for the steady-state model is in a tabular form only, whereas solutions for the dynamic model can take the form of tables or concentration-time curves for various compartments. The overall persistence of the system is also calculated.

Key References:

Asher, S.C., K.M. Lloyd, D. Mackay, S. Paterson and J.R. Roberts. 1985. A critical examination of environmental monitoring-modeling the fate of chlorobenzenes using the persistence and fugacity models. Rep. No. NRCC 23990. National Research Council of Canada.

Roberts, J.R., M.S. Mitchell, M.J. Boddington and J.M. Ridgeway. 1981. A screen for the relative persistence of lipophilic organic chemicals in aquatic ecosystem- An analysis of the role of a simple computer model in screening, Part I. National Research Council of Canada. Rep. No. NRCC 18570, Ottawa, Canada.

Logistical Considerations:

Equipment: Mainframe

Critique/Comments: Requires measures or estimates of photodegradation, volatilization and hydrolysis in water; biodegradation in fish.

Model Name: PIRAHNA (Pesticide and Industrial Chemical Risk Analysis and Hazard Assessment, version 2.0)
Model Type: Fate, Bioaccumulation, Databases

Description:

PIRAHNA is an ecological risk tool designed for analysts who have environmental safety responsibilities for synthetic chemicals. It is a vehicle for transmittal of results of the U.S. EPA's Office of Research and Development (ORD) Ecological Risk Assessment ("EcoRisk") Research program to the user community-- EPA and State regulatory scientists, and industrial chemical safety specialists. The documentation for PIRAHNA is being released in annual updates over the period from 1990 to 1995. The final version will encompass analytical capabilities ranging from the estimation of chemical properties from chemical structure through risks attending chemical releases to whole ecosystems.

Key References:

Burns, L.A., B.W. Allen, Jr., M.C. Barber, S.L. Bird, J.M.Cheplick, D.R. Hartel, C.A. Kittner, F.L. Mayer, L.A. Suarez and S.E. Wooten. 1991. PIRAHNA, version 2.0.

Logistical Considerations:

Equipment: IBM compatible PC

Critique/Comments: PIRAHNA is composed of three models described individually in this chapter (PRZM, EXAMSII,FGETS). PIRAHNA also includes agricultural crop census and ichthyolfaunal geographic range databases.

Model Name: QWASI
Model Type: Fate, Multimedia, Fugacity

Description:

The model was developed by Mackay et al. (1983) to treat the fate of a chemical discharge to a water-air-sediment system using Z and D values, volumes, areas, flows and the input parameters to give a steady-state algebraic solution. Algebraic and numerical time-dependent solutions can be written by the user. The program is not "user-friendly". The conditions simulated in the program are similar to those described by Mackay (1989) for the fate of PCBs in Lake Ontario.

Key References:

Mackay, D. 1989. Modeling the long term behaviour of an organic contaminant in a large lake: Application to PCBs in Lake Ontario. J. Great Lakes Res. 15:283-297.

Mackay, D., S. Joy and S. Paterson. 1983. A quantitative water air sediment interaction (QWASI) model for describing the fate of chemicals in lakes. Chemosphere 14:335-374.

Logistical Considerations:

Equipment: IBM compatible PC

Critique/Comments: The user must specify conditions by editing the appropriate lines of code. Some users find it more convenient to write the equations into a spreadsheet, such as Lotus 1-2-3 or Quattro Pro, from which the results can be plotted directly.

Model Name: SIMPLESAL
Model Type: Fate, Exposure, Multimedia, Fugacity

Description:

This is a spreadsheet-based model which can be used to estimate steady-state or time-dependent concentrations of organic compounds as well as heavy metals. It determines dominant environmental pathways and processes for contaminants, and was designed for use in the Netherlands as a screening tool to predict results of various scenarios for emission control of new and existing chemicals such as benzene, cadmium, lindane and copper. It considers air, water, suspended solids, aquatic biota, sediment and soil compartments.

The model incorporates processes of advective flows, diffusive and non-diffusive transfer, bioconcentration in aquatic biota, leaching from soil to groundwater, and biotic and abiotic transformation.

Key References:

OECD (Organization for Economic Cooperation and Development). 1989. Compendium of Environmental Exposure Assessment Methods for Chemicals. Environment Monographs, No. 27, OECD, Paris.

Logistical Considerations:

Equipment: Mainframe

Critique/Comments: Data required to run the model include dimensions, properties of and emissions into environmental media; air and water residence times; parameters for intercompartment transfer in association with particulates; physico-chemical propties of water solubility, vapor pressure and octanol-water partition coefficient and degradation rate constants.

Model Name: Spatial Multimedia Compartmental Model (SMCM)
Model Type: Fate, Multimedia

Description:

The model was developed by the National Center for Intermedia Transport at UCLA. It describes the fate of chemicals in a conventional air-water-soil-sediment system under steady-state or dynamic conditions. It allows for concentration variation with depth in the soil or sediment.

Key References:

Cohen, Y. 1989. The Spatial Multimedia Compartments Model (SMCM), User's Manual Version 3.0, NCITR, UCLA, CA

Cohen, Y., Tsai, W., S.L. Chetty and G.L. Mayer. 1990. Dynamic partitioning of organic chemicals in regional environments: A multimedia screening-level modeling approach, Environ. Sci. Technol. 24:1549-1558.

Logistical Considerations:

Training: Software can be run with virtually no background in transport phenomena.

Equipment: IBM compatible PC

Critique/Comments: The model is user friendly with help menus and the capacity of presenting data output in tabular or graphical form.

Model Name: Toxscreen
Model Type: Fate, Multimedia

Description:

Toxscreen is a time-dependent multimedia model, developed by the U.S. EPA to assess the potential for environmental transport and accumulation of chemicals released into the air, surface water or soil. It is modular in concept and incorporates intermedia transfer processes. It is a screening tool to assess the potential for human exposure to chemicals.

Atmospheric dispersion is incorporated into the model using a Gaussian plume dispersion model. Contaminant migration in soil following direct application and transport between other media is estimated by means of the SESOIL model. Pollutant concentrations in water bodies are estimated over a period of time using a method similar to the EXAMS model.

Key References:

Hetrick, D.M. and L.M. McDowell-Boyer. 1983. User's manual for TOXSCREEN: A multimedia screening level program for assessing the potential fate of chemicals released into the environment. ORNL/TM-8570. Oak Ridge National Laboratory, Oak Ridge, TN.

Logistical Considerations:

Equipment: Mainframe

Critique/Comments: Data requirements are extensive, including information on the characteristics of the environment treated and emission sources; chemical and degradation parameters; and climatological, meteorological and hydrological parameters. Data files on climatic and soil conditions for some regions are included with the model. The output is in the form of an estimation of the fate of a chemical over a period of time.

Model Name: WQAM (Water Quality Assessment Methodology)
Model Type: Surface Water Fate

Description:

This is a steady state, one dimensional model; requiring only desktop calculations. It provides canonical information. The methods are useful for modeling lakes, rivers and estuaries.

Key References:

Mills, W.B., J.D.Dean, D.B. P.B. Porcella et al. 1982. Water quality assessment: a screening procedure for toxic and conventional pollutants: parts 1, 2 & 3. Athens, GA: USEPA. Environmental Research Laboratory. Office of Research and Development. EPA 600/6-82/004 a,b,c.

Logistical Considerations:

Equipment: Hand calculator.

Critique/Comments: Recommended if time, costs or information are restrictive.

Model Name: SLSA (Simplified Lake/Stream Analysis)
Model Type: Surface Water Fate

Description:

This is a steady state, one dimensional model. It may be solved by desk top calculation or via a simple computer program in languages such as Fortran, Pascal or BASIC or through calculations in a spreadsheet. It is suited to simplified lake or river systems.

Key References:

Hydroqual, Inc. 1982. Application guide for CMA-Hydroqual chemical fate models. Prepared for: Chemical Manufacturer's Association, Washington, DC. As reviewed in: Versar Inc. 1983. Methodology for assessing exposures to chemical substances via the ingestion of drinking water. Washington,DC: US EPA contract No. 68-01-6271.

Logistical Considerations:

Equipment: Hand calculator or IBM compatible PC

Critique/Comments: Well documented and suggested for use prior to use of a more sophisticated model.

Model Name: MICHRIV (Michigan River Model)
Model Type: Surface Water Fate

Description:

This is a steady state, one dimensional model. It is a computer program written in Fortran. It is similar to SLSA, but is capable of handling more than one reach. It is intended for modeling metals fate and is suitable for rivers and streams.

Key References:

Delos, C.G., W.L. Richardson, J.V. DePinto et al. 1984. Technical guidance manual for performing wasteload allocations, book II: streams and rivers. USEPA. Office of Water Regulations and Standards. Water Quality Analysis Branch. Washington, DC. (Draft Final).

Logistical Considerations:

Equipment: IBM compatible PC

Critique/Comments: Well documented and suggested for use prior to use of a more sophisticated model. It is easy to set up and use and requires minimal computer programming.

Model Name: SARAH (Surface Water Back Calculation Procedure)
Model Type: Contaminant Fate in Surface Waters.

Description:

This is a steady state, one dimensional analytical solution coded in Fortran. It is designed for simulation of contaminated leachate plume feeding the down gradient surface waterbody (stream or river). The solution is generated from a Monte Carlo simulated generic environment. It considers degradation, volatilization, dilution and sorption, and bioaccumulation in fish.

Key References:

Jan. 14, 1986 Federal Register, Hazardous Waste Management System, Land Disposal Restrictions, Proposed Rule

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Logistical Considerations:

Equipment: Mainframe.

Critique/Comments: The model requires mimimal data input. Data on degradation, volatilization, dilution and sorption, and bioaccumulation in fish may be estimated by model default parameters.

Model Name: SESOIL (Seasonal Soil Compartment Model)
Model Type: Unsaturated Zone and Groundwater Fate

Description:

This is an integrated screening level soil compartment model designed to simultaneously model water transport, sediment transport and pollutant fate. It was developed for the EPA offices of Water Quality, and Toxic Substances (OTS). The model was originally coded in fortran but has since been encoded in PCGEMS (Graphical exposure modeling for the PC). It is a complete information management tool developed for EPA-OTS and designed to help users perform exposure assessments. PCGEMS has subsequently been transformed into a system called RISKPRO, which is an upgraded PCGEMS system.

The model may be used to simulate chemical releases to soil from sources such as landfill disposal, accidental leaks, agricultural applications, leaking underground storage tanks, or deposition from the atmosphere. Potential applications include long-term leaching from waste disposal sites, pesticide and sediment transport on watersheds, studies of hydrologic cycles on watersheds and water balances of soil compartments. The effect of site management or design strategies on pollutant distributions and concentrations in the environment may also be simulated.

Key References:

Bonazountas, M. and J. Wagner. 1981. SESOIL, a seasonal soil compartment model. Cambridge, MA: A.D. Little, Inc. for USEPA. Contract No. 68-01-6271.

Logistical Considerations:

Programming Language: Fortran

Equipment: PC-GEMS system (or RISKPRO) on a IBM compatible PC, VAX 11/780, IBM 370

Critique/Comments: Versatile, easy to use.

Model Name: LPMM (Leachate Plume Migration Model)
Model Type: Unsaturated Zone and Groundwater Fate

Description:

This is a continuous source model that simulates dispersion from the a source. Degradation mechanisms are taken into account by the model. It is a simplistic model useful as a screening tool, but not for level III work.

Key References:

Kent, D.C., W.A. Pettyjohn, F. Witz and T.A. Prickett. 1982. Prediction of leachate plume migration and mixing in groundwater. Solid and Hazardous Water Research and Development Annual Symposium proceedings. Columbus, OH: National Water Well Association. As reviewed in: Versar, Inc. 1983. Theoretical evaluation of sites located in the zone of saturation. Draft final report. Chicago, IL: USEPA. Contract No. 68-01-6438.

Logistical Considerations:

Programming Language: must be written by user.

Equipment: Handheld calculator or computer.

Critique/Comments: The model has been field-verified and is easy to use.

Model Name: RAPS (Remedial Action Priority System)
Model Type: Fate, Remediation

Description:

This system was developed by the U.S. Department of Energy to set priorities for investigation and possible cleanup of chemical and radioactive waste disposal sites. It is intended to be used in a comparative rather than predictive manner. The methodology considers four major pathways of contaminant migration: groundwater, surface water, overland, and atmospheric. Estimated concentrations in the air, soil, sediments, and water media are used to assess exposure to neighboring populations.

Data used to run RAPS includes on site and pollutant characteristics to simulate migration and fate from source to receptor by various pathways.

The estimated environmental concentrations form the basis of subsequent human exposure calculation and determination of the Hazard Potential Index (HPI). The modular development makes it useful for the inclusion of additional components.

Key References:

Whelan, G., D.L. Strenge, J.G. Droppo, B.L. Steelman and J.W. Buck. 1987. The remedial action priority system (RAPS): Mathematical Formulations, PNL-6200. Pacific Northwest Laboratory, Richland, WA.

Logistical Considerations:

Equipment: Mainframe

Critique/Comments: The model methods are not truly multimedia, because it is based on use of independent modules which do not interact spatially or temporally; transfer of a pollutant is unidirectional. This modular framework permits updating of, or inclusion of, addition components with improvement of technology. The user supplies appropriate routes of chemical from waste site to neighboring populations through various media including air, groundwater, soil and vegetation.

Model Name: RWSTM (Random Walk Solute Transport Model; aka TRANS)
Model Type: Unsaturated Zone and Groundwater Fate

Description:

This is a one-dimensional or two-dimensional model accounting for time-variant release rates. The model accomodates well injected releases, incorporates dispersion and retardation and accounts for well pumping. It is capable of providing estimates of nonconservative pollutant concentrations at user selected points.

Key References:

Prickett, T.A., T.G. Naymik and C.G. Lonnquist. 1981. A "random-walk" solute transport model for selected groundwater quality evaluations. Champaign, IL: Illinois Department of Energy and Natural Resources. ISWS/BUL-65/81. As reviewed in: Versar Inc. 1983. Theoretical Evaluation of sites located in the zone of saturation. Draft Final Report. Chicago, IL: USEPA. Contract No. 68-01-6438.

Logistical Considerations:

Equipment: Mainframe

Critique/Comments: Model use requires mathematical programming and hydrogeological knowledge on the part of the user.

Model Name: CFEST (Coupled Fluid, Energy and Solute Transport) combined with UNSAT-ID
Model Type: Unsaturated Zone and Groundwater Fate

Description:

This is a three-dimensional model combination accommodating heterogeneous, anisotropic, multilayered soil configurations. The model combination can be utilized for saline and freshwater aquifers. Dispersive and advection transport mechanisms are simulated by the models; sorption and degradation mechanisms are not. It can be used in unsaturated and saturated zones for simulation of time-variant releases and flow rates.

Key References:

Gupta, S.K., C.R. Cole, C.T. Kincaid and A.M. Monti. 1987. Coupled fluid, energy and solute transport (CFEST) model: formulation and user's manual. Columbus, Ohio: Office of Nuclear Waste Isolation, Battelle Memorial Institute.

Logistical Considerations:

Equipment: Mainframe

Critique/Comments: The model combination has been applied to arsenic and organic wastes.

Model Name: SWIFT and SWIFT II (Sandia Waste Isolation Flow and Transport Model
Model Type: Unsaturated Zone and Groundwater Fate

Description:

This is a three-dimensional model accommodating heterogeneous, anisotropic, multilayered soil configurations. The model combination can be utilized for saline and freshwater aquifers. Dispersive and advection transport mechanisms are simulated by the models. Sorption and degradation mechanisms are also taken into account. It is appropriate for use in waste-injection, waste-isolation simulation.

Key References:

Finley, N.C. and M. Reeves. 1968. SWIFT self-teaching curriculum. Washington, DC: Nuclear Regulatory Commission. NUREG/CR-1968, SAND 81-0410. As reviewed in: Lo T.Y.R., B.H. Scott and R.R. Benjamin. 1983. Remedial action assessment models for hazardous waste sites. Review draft. Athens, GA: USEPA. Contract No. 68-03-3116.

Reeves, M. and R.M. Cranwell. 1981.User's manual for the Sandia Waste-Isolation Flow Transport Model (SWIFT). Washington, DC: Nuclear Regulatory Commission. NUREG/CR-2324, SAND 81-2516. As reviewed in: Lo T.Y.R., B.H. Scott and R.R. Benjamin. 1983. Remedial action assessment models for hazardous waste sites. Review draft. Athens, GA: USEPA. Contract No. 68-03-3116.

Software: National Energy Software Center
Argonne National Laboratories
Argonne, IL 60439

Logistical Considerations:

Programming Language: Fortran

Equipment: Has been used on CDC systems, including CDC 7600

Critique/Comments: The model has been field-verified. It comes with a user's guide written in a self-teaching format.

Model Name: CTAP (Chemical Transport and Analysis Program)
Model Type: Surface Water Fate

Description:

This is a steady state, three dimensional compartmental model. It is a computer program written in Fortran IV and suitable for numerous personal computers. It is similar to SLSA except more sophisticated. Each component of the model is equilavalent to one SLSA lake. It is intended for modeling fate in streams, stratified rivers, lakes, estuaries and coastal embayments.

Key References:

Hydroqual, Inc. 1982. Application guide for CMA-Hydroqual chemical fate models. Prepared for: Chemical Manufacturer's Association, Washington, DC. As reviewed in: Versar Inc. 1983. Methodology for assessing exposures to chemical substances via the ingestion of drinking water. Washington,DC: US EPA contract No. 68-01-6271.

Logistical Considerations:

Equipment: Suitable for IBM 360/370, UNIVAC 108, CDC 6600 mainframe computers and IBM compatible PC

Critique/Comments: Well documented and suggested for use following use of a less sophisticated model. The model requires extensive data input.

Model Name: HPSF (Hydrological Simulation Program-FORTRAN)
Model Type: Surface Water Fate

Description:

This is a time varying, one dimensional model. It is a computer program written in Fortran and suitable for numerous personal computers. It is designed for year-round simulation of organic pollutant fate in non-tidal rivers, streams and mixed lakes according to a second order decay mechanism.

Key References:

Johanson, R.C., G.C. Imhoff and H.H. Davis. 1984. Hydrocomp Inc. User's manual for hydrological simulation program - Fortran (HSPF). Athens, GA: Office of Research and Development, USEPA. EPA-600/9-80-015. As reviewed in: Versar, Inc. 1983. Methodology for assessing exposures to chemical substances via the ingestion of drinking water. Washington, DC: USEPA. Contract No. 68-01-6271.

Logistical Considerations:

Equipment: IBM compatible PC. Can be used on mainframes.

Critique/Comments: The model requires extensive data input. The software is available from the Center for Exposure Assessment Modeling (CEAM), Environmental Research Laboratory, U.S. EPA Athens, GA 30613. Their phone number is (706) 546-3130/ fax (706)-546-2018.

Model Name: TODAM (Transient One-Dimensional Degradation and Migration Model)
Model Type: Surface Water Fate

Description:

This is a time varying, one dimensional model. It is designed for simulation of second order decay processes in river and estuarine systems. The model requires use of an exterior hydrodynamic model to provide channel and flow velocities.

Key References:

Onishi, Y., G. Whelan and R.L. Skaggs. 1982. Development of multimedia radionuclide exposure assessment methodology for low-level waste management. Athens, GA: Office of Research and Development, USEPA. As reviewed in: Versar Inc. 1983. Methology for assessing exposures to chemical substances via the ingestion of drinking water. Washington, DC: USEPA. Contract No. 68-01-6271.

Logistical Considerations:

Equipment: VAX or PDP 11/70

Critique/Comments: The model requires extensive data input. It is a complex FORTRAN computer program written in the preprocessor language, FLECS, or in Fortran IV.

Model Name: CHNTRN (Channel Transport Model)
Model Type: Surface Water Fate

Description:

This is a time varying, one dimensional model. It is a complex FORTRAN IV computer program. It is designed for simulation of second order decay processes of organic pollutants in rivers, lakes, estuaries and coastal waters. The model can be coupled to a hydrodynamic model, CHNHYD, to estimate flow dynamics where such data are not available.

Key References:

Yeh, G.T. 1982. CHNTRN: a chemical transport model for simulating sediment and chemical distribution in a stream/river network. Washington, DC: Office of Pesticides and Toxic Substances, USEPA. Contract No. W-7405-eng-26. As reviewed in: Versar 1983. Methodology for assessing exposures to chemical substances via the ingestion of drinking water. Washington, DC: USEPA. Contract No. 68-01-6271.

Logistical Considerations:

Equipment: IBM 3933 and others.

Critique/Comments: The model requires extensive data input and has an extensive set up time.

Model Name: FETRA (Finite Element Transport Model)
Model Type: Surface Water Fate

Description:

This is a time varying, two dimensional (longitude and latitude) model. It is designed for simulation of second order decay processes of organic pollutants in rivers, estuaries, coastal systems and completely mixed lakes. The model can be coupled to a hydrodynamic model, EXPLORE, to estimate flow dynamics where such data are not available.

Key References:

Onishi, Y. 1981. Sediment-contaminant transport model. Journal of the Hydraulics Division, ASCE. 107(HY9):1089-1107. Proc. Paper 16505. As reviewed in: Versar Inc. 1983. Methodology for assessing exposures to chemical substances via ingestion of drinking water. Washington, DC: USEPA. Contract No. 68-01-6271.

Logistical Considerations:

Equipment: IBM, VAX or CDC-7600 computers.

Critique/Comments: It is written in FORTRAN IV computer programming language. The model requires extensive data input and has extensive setup and execution time requirements.

Model Name: WASP4 (Estuary and Stream Quality Model)
Model Type: Surface Water Fate

Description:

This is a time varying, three dimensional model. It is designed for simulation of second order decay kinetics of organic pollutants in rivers, lakes and estuaries.

Key References: Unavailable

Logistical Considerations:

Equipment: IBM 370 or PDP-11/70

Critique/Comments: The user must provide hydrodynamic flows between model compartments. The model requires extensive data input and has extensive setup and execution time requirements. The software is available from the Center for Exposure Assessment Modeling (CEAM), Environmental Research Laboratory, U.S. EPA Athens, GA 30613. Their phone number is (706) 546-3130/ fax (706)-546-2018.

Model Name: Sediment Chronology Models
Model Type: Fate, Aquatic

Description:

Bottom sediments serve as sinks for many metallic and hydrophobic contaminants. These models typically assume the possibility of estimating the historic condition of a lake by examining the variation of contaminant concentration with depth of burial. Considerable effort has been devoted to deducing the likely fate of buried chemicals when subject to diagenetic processes. These efforts usually take the form of multilayer models in which the year by year transport and transformation rates are estimated in slices of buried sediment. Examples of studies of this type are exemplified in Hites and Eisenreich (1987).

Key References:

Hites, R.A. and S.J. Eisenreich (eds.). 1987. Sources and Fates of Aquatic Pollutants. Advances in Chemistry Series 216, ACS, Washington, D.C.

Logistical Considerations:

Equipment: Personal computer, micro-computer

Critique/Comments: Horizontal movement of contaminants should be considered when applying this type of model.

Model Name: PRZM (Pesticide Root Zone Model)
Model Type: Unsaturated Zone and Groundwater Fate, Vertical Runoff

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Description:

PRZM is a dynamic compartmental model for use in simulating chemical movement in the unsaturated soil systems within and below the plant root zone. It is capable of simulating movement of potentially toxic chemicals, particularly pesticides, that are applied to soil or to plant foliage as pulse loads, predicting peak events and estimating time-varying mass emissions or concentration profiles.

There are three major components to the model: (a) water movement, (b) soil erosion and (c) chemical transport and transformation.

Key References:

Carsel,R.F., C.N. Smith, L.A. Mulkey, J.D. Dean and P. Jowise. 1984. User's manual for the pesticide root zone model (PRZM) Release 1. USEPA EPA-600/3-84-109. U.S. Gov't Printing Office.

Williams, J.R. 1975. Sediment yield predictions with universal equation using runoff energy factor. In: Present and prospective technology for predicting sediment yields and sources. USDA-ARS, U.S. Gov't Printing Office. pp. 244-252.

Logistical Considerations:

Training: PIC is designed to be easily assessible to the novice user.

Time: Basic understanding of the system can be gained within a day.

Equipment: Can be run utilizing a PC equipped with a math coprocessor.

Critique/Comments: The current state of model utilization is through the PRZM Input Collator (PIC), which provides an interface between PRZM and a series of databases to allow efficient generation and modification of PRZM input data sets. PIC also contains utilities to allow the user to explore the data bases and screen geographically based information. The software is available from the Center for Exposure Assessment Modeling (CEAM), Environmental Research Laboratory, U.S. EPA Athens, GA 30613. Their phone number is (706) 546-3130/ fax (706)-546-2018.

Model Name: SERATRA (Sediment-Contaminant Transport)
Model Type: Surface Water Fate

Description:

This is a time varying, two dimensional (longitudinal and vertical) model that accounts for complex sediment transport mechanisms. It is designed for simulation of second order decay processes of organic pollutants in rivers and lakes.

Key References:

For documentation: ORD Publications. Center for Environmental Research, Information, USEPA, Cincinnati, OH 45268 (513)-546-7562.

Onishi, Y. and S.E. Wise. 1982. Mathematical model, SERATRA, for sediment-contaminant transport in rivers and its applicaion to pesticide transport in Four Mile and Wolf creeks in Iowa. Athens, GA: Office of Research and Development, USEPA. EPA-600-3/82-045. As reviewed in: Versar Inc. 1983. Methodology for assessing exposures to chemical substances via ingestion of drinking water. Washington, DC: USEPA. Contract No. 68-01-6271.

Onishi, Y. and S.E. Wise. 1982. User's manual for instream sediment-contaminant transport model SERATRA. Athens, GA: Office of Research and Development, USEPA. EPA-600/3-82-055. As reviewed in: Versar Inc. 1983. Methodology for assessing exposures to chemical substances via ingestion of drinking water. Washington, DC: USEPA. Contract No. 68-01-6271.

Logistical Considerations:

Equipment: Vax, mainframe

Critique/Comments: It is written in FORTRAN preprocessor language FLECS< in batch mode. It has been field tested and is available for use. The model requires extensive data input and has extensive setup and execution time requirements. It is estimated to require a person 750 hours to prepare the model to run, assuming all data are readily available.

Model Name: CMLS (Chemical Movement in Layered Soils) Ver. 4.2
Model Type: Unsaturated/Saturated Flow, Chemical Fate in Soils

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Description:

CMLS is an interactive microcomputer model. It was written to serve as a management tool and decision aid for the application of organic chemicals to soils. CMLS is an integrated soil compartment model designed to simultaneously model water and chemical transport, evapotranspirative effects, and the fate of non-polar organic chemicals. The model estimates the location of the peak concentration of the chemicals as they move through a soil in response to downward movement of water, and the relative amount of each chemical still remaining in the soil at any time. The model can handle soils with up to 20 different layers or horizons, so soil properties need not be assumed uniform by depth. Different partition coefficients and degradation half-lives for the chemicals of interest can be designated for each layer within the soil, to account for differing chemical/soil property interactions. Chemical movement and degradation can be simulated for up to 15 years. Results may be displayed in graphical as well as tabular form. The user may also request output designating the amount of time required for selected chemicals to move to user-specified depths within the soil profile.

Key References:

Nofziger, D.L. and A.G. Hornsby. 1987. Chemical Movement in Layered Soils: User's Manual. Circular 780, Computer Series, Software in Soil Science, Florida Cooperative Extension Service. Institute of Food and Agicultural Sciences, University of Florida, Gainesville, FL.

Logistical Considerations:

Training: Software can be run on the basis of the information provided in the user's manual, however selecting appropriate input for the model requires knowledge of soil science and/or transport phenomena.

Equipment: Minimal requirements for CMLS Ver. 4.2 are an IBM compatible XT microcomputer with 512K bytes

of random access memory (RAM), two disk drives, and enhanced graphics array (EGA). Operating system must be DOS, version 2.0 or later. A printer and math coprocessor are beneficial.

Data Requirements:

For each soil layer/horizon
- depth
- percent organic carbon (%OC)
- soil bulk density
- water content at -0.01 -1.5 MPa, and saturation

For each chemical
- partition coefficient
- degradation half-life

Max. rooting depth of plants at soil surface
Initial depth of chemicals
Daily effective precipitation and evapotranspiration records

Critique/Comments: Well documented, interactive, flexible and user-friendly. Model may be used for simple or more sophisticated modeling. CMLS is especially useful for modeling situations where diverse soil horizons or soil properties exist.

Model Name: LEACHM (Leaching Estimation And CHemistry Model)
Model Type: Unsaturated Zone, Chemical Fate in Soils

Description:

LEACHM is a process-based model that simulates water and solute movement in soils, chemical transformations and fate, plant uptake, and chemical reactions in the vadose zone. LEACHM is a general acronym that refers to three linked simulation models that describe the chemistry, transport, sorption, degradation, and volatilization of chemical compounds in the plant root zone and through the soil profile, with the three linked models sharing the same numerical solution scheme to simulate water and chemical transport. Chemical fate is determined in response to a variety of environmental parameters including soil characteristics; precipitation, evaporation, and transpiration of water; uptake of solutes and plant growth; and heat flow (temperature profiles). LEACHM includes the flexibility of simulating layered or non-homogeneous soil profiles. The three linked models consist of LEACHMP, LEACHMS, and LEACHMN. LEACHP simulates the chemistry, transport, and degradation of organic compounds, pesticides (P), in soils. LEACHMS is formulated to describe transient movement of inorganic salt solutes (S) (including Ca, Mg, Na, K, SO_4, Cl, CO_3, and HCO_3) and corresponding soil chemical reactions. LEACHMN is organized to describe the transport and transformation of nitrogen (N) in soils.

Key References:

Wagenet, R.J. and J.L. Hutson. 1987. LEACHM: Leaching Estimation And CHemistry Model - A process-based model of water and solute movement, transformations, plant uptake and chemical reactions in the unsaturated zone. CONTINUUM, Vol. 2, Water Resources Institute. Center for Environmental Research, Cornell University, Ithaca, NY.

Hutson, J.L. and R.J. Wagenet. 1988. Leaching Estimation And CHemistry Model: LEACHM - A user's guide. Department of Agronomy, Cornell University, Ithaca, NY.

Logistical Considerations:

Programming Language: FORTRAN

Training: LEACHM is a sophisticated and relatively complex model. Before running the software, the user must read the references cited above and have a working knowledge of soil science and transport phenomena.

Equipment: Microsoft FORTRAN77 compiler (Ver. 3.2), on an IBM compatible PC operating under MS-DOS. A printer and math coprocessor are recommended.

Data Requirements:

Starting and last day nos.
Number of soil segments (layers/horizons)
Number of depth nodes (sub-segments)
Largest time interval for modeling
Max. theta (water) change/time step
Min. time interval/day
No. of water applications
No. of pesticide (organic chemical) applications
No. of fertilizer applications
Initial depth and concentrations
Initial theta (volumetric water content)
Initial temperature
Initial matric potential
Air entry value (AEV)
Selectivity coefficients for ion exchange
Pan evaporation

Water application and composition
- time
- amount
- rate

If plant cover is specified
- planting time (day)
- seedling emergence (day)
- plant maturity (day)
- mature root profile (day)
- harvest (day)
- roots (constant, or growing)
- relative root distribution (fraction)
- crop cover at maturity (fraction)
- min. and max. root zone water potential

- root flow resistance
- max. actual transpiration/potential transpiration

For each soil layer/horizon
- depth
- percent organic carbon (%OC)
- soil bulk density
- hydraulic conductivity value
- bottom boundary condition

For each chemical
- partition or distribution coefficient
- molecular diffusion coefficient
- empirical diffusion constant values (a and b)
- dispersivity
- saturated vapor density
- water solubility
- Campbell's exponent for retentivity
- initial concentration
- transformation/degradation products
- initial concentrations trans./degrad. prods.
- degradation half-life
- transformation constant

Critique/Comments: Well documented, complex but flexible. Assistance running the model is available from co-author John L. Hutson (telephone: (607) 255-7631), Cornell University. Model is recommended for more sophisticated quantitative modeling of chemical fate. LEACHM is especially useful for modeling situations where diverse soil horizons or soil properties exist and there is substantial supporting soil/environmental data.

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Technique Name:	Cytochrome P450 Induction
Technique Type:	Enzyme Induction
Matrix Type:	Biological Tissue
Ecosystem Level:	Individual
Test Location:	Laboratory

Description:

This technique can be used to analyze the induction of enzymes such as cytochrome P-450IIB (phenobarbital type) and P-450IA (3-methylcholanthrene type) in the liver and kidney. These enzymes are involved in the oxidative metabolism of such compounds as fatty acids, steroids, prostaglandins, leukotrienes, biogenic amines, pheromones, and plant metabolites. Further, cytochrome P-450s metabolize innumerable drugs, chemical carcinogens, mutagens, and other environmental contaminants. In one assay used to detect the induction of cytochrome P450s, liver or kidney samples are taken from environmentally exposed individuals and are processed to create microsomes, which contain the membrane-bound cytochrome P-450s. The amount of cytochrome P-450s present in the sample can be assessed by measuring fluroescence after exposing microsomes to ethoxyresorufin-O-deethylase (EROD) or pentoxyresorufin-O-deethylase activity (PROD), two enzymes which fluoresce in the presence of specific forms of cytochrome P-450s.

Logistical Considerations:

Sample Collection:

Training: MODERATE

Time: MODERATE

Equipment: Liquid nitrogen, liquid nitrogen storage container - Samples must be stored at -80°C immediately after collection until microsomes can be prepared.

Sample Analysis:

Training: MODERATE.

Time: MINIMAL to run an individual sample, MODERATE to run larger numbers of samples

Equipment: Centrifuge, ultracentrifuge, fluorometer, spectrophotometer, 96-well plate reader for fluorimeter, 96-well plate reader for spectrophotometer, -80° C freezer

Critique/Comments:

After extraction, organs from exposed individuals must be frozen at temperatures less than -80° C immediately after the individual is removed from the contaminated site.

Results of this test must be interpreted cautiously because factors other than exposure to anthropogenic materials can induce cytochrome P-450s. These factors include naturally occurring plant toxins.

Key References:

Hofius, J.L. 1992. Characterization and induction of hepatic and renal detoxification enzymes in nestling European starlings (*Sturnus vulgaris*). Master of Science Thesis unpublished. Clemson University, Clemson, SC.

Nebert, D.W. and F.J. Gonzalez. 1987. P450 genes: structure, evolution, and regulation. Ann. Rev. Biochem. 56:945-993.

Payne, J.F., L.L. Fancey, A.D. Rahimutula and E.L. Porter. 1987. Induction of hepatic mixed function oxidases in the Herring gull (*Laurus argentatus*) by Prudhoe Bay crude oil and its fractions. Comp. Biochem. Physiol. 94(C):461-463.

Rattner, B.A., D.J. Hoffman and C.M. Marn. 1989. Use of mixed-function oxygenases to monitor contaminant exposure in wildlife. Environ. Toxic. Chem. 8:1093-1102.

Simmons, G.J. and M.J. McKee. 1992. Alkoxyresorufin metabolism in white-footed mice at relevant environmental concentrations of Aroclor 1254. Fund. Appl. Toxicol. 19:001-006.

Walters, P., S. Kahn, P.J. O'Brien, J.F. Payne and A.D. Rahimtula. 1987. Effectiveness of a Prudhoe Bay crude oil and its aliphatic, aromatic, and heterocyclic fractions in inducing mortality and aryl hydrocarbon hydroxylase in chick embryo in *ovo*. Arch. Toxicol. 60:454-459.

Technique Name: Cholinesterase Inhibition

Technique Type: Enzyme Inhibition
Matrix Type: Biological Tissue
Ecosystem Level: Individual
Test Location: Field, Laboratory

Description:

In unstressed cells, cholinesterases are responsible for the degradation of neurotransmitters. Inhibition of these enzymes causes a variety of neurotoxic responses in invertebrates, birds, and fish. Various assays have been developed to measure cholinesterase activity, and normal activity levels have been established for several species of birds and mammals. The role of organophosphate and carbamate pesticides in the inhibition of acetylcholinesterase has been extensively researched. Other contaminants, including mercury, have been shown to cause inhibition.

Logistical Considerations:

Sample Collection:

Training: MODERATE

Time: MODERATE

Equipment: Samples need to be placed on wet ice immediately after collection to prevent enzyme reactivation. Samples stored for periods >8h should be stored at tempertures < -20°C.

Sample Analysis:

Training: MODERATE

Time: MINIMAL

Equipment: A spectrophotometer is required for sample analysis.

Critique/Comments:

Data reported in the literature suggests there may be variation in baseline ChE values within a population, and variation among seasons. Reactivation by 2-pyridine aldoxime methiodide (2-PAM) seems to occur infrequently, but is a strong indicator of exposure when it is found. Collecting the samples is relatively simple with training personnel and analysis costs are relatively inexpensive. Cholinesterase analyses of blood plasma can be repeatedly collected from the same individual over time.

Key References:

Ellman, G.L., K.D. Courtney, V. Andres and R.M. Featherstone. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7:88-95.

Fairbrother, A., B.T. Marden, J.K. Bennett and M.J. Hooper. 1991. Methods used in determination of cholinesterase activity, In P. Mineau, ed., Cholinesterase-inhibiting Insecticides. Elsevier Science Publishers, Amsterdam, pp.35-71.

Grue, C.E., W.J. Fleming, D.G. Busby and E.F. Hill. 1983. Assessing hazards of organophosphate pesticides to wildlife. Trans. N. Am. Wildl. Nat. Res. Conf. 48:200-220.

Grue, C.E., G.V.N. Powell and N.L. Gladson. Brain cholinesterase (ChE) activity in nestling starlings: implications for monitoring exposure of nestling songbirds to ChE inhibitors. Bull. Environ. Contam. Toxicol. 26:544-547.

Hill, E.F. and W.J. Fleming. 1982. Anticholinesterase poisoning of birds: field monitoring and diagnosis of acute poisoning. Environ. Toxicol. Chem. 1:27-38.

Jett, D.A. 1986. Cholinesterase inhibition in meadow voles (*Microtus pennsylvanicus*) following field applications of Orthene. Environ. Toxicol. Chem. 5:255-259.

Technique Name: Porphyrin Profiles

Technique Type: Enzyme Inhibition
Matrix Type: Biological Tissue
Ecosystem Level: Individual
Test Location: Laboratory

Description:

This technique assesses levels of intermediates in the heme synthesis pathway in tissues, blood, and excreta of mammals, birds, and aquatic organisms. Research to date has indicated this methodology provides both qualitative and quantitative biomarkers of exposure to toxicants such as polyhalogenated hydrocarbons and heavy metals. Levels may be measured in liver, kidney, and fecal-urate excreta. Measurements are made of 8-, 7-, 6-, 5-, 4-, and 2-carboxyl porphyrin concentrations.

Logistical Considerations:

Sample Collection:

Training: MODERATE

Time: MODERATE

Equipment: Liquid nitrogen, liquid nitrogen storage container - Samples must be stored at -80°C immediately after collection.

Sample Analysis:

Training: MODERATE

Time: MINIMAL for individual samples.

Equipment: Spectrofluorometer and HPLC are both needed for analysis.

Critique/Comments:

Sample storage temperature is critical since porphyrin intermediates rapidly degrade after sacrifice of the specimen.

Key References:

Akins, J.M., M.J. Hooper, H.D. Miller and J.S. Woods. 1993. Porphyrin profiles in the nestling European starling (*Sturnus vulgaris*): a potential biomarker of field contaminant exposure. Journal of Toxicology and Environmental Health 40:47-59.

Bowers, M.A., L.D. Aicher, H.A. Davis and J.S. Woods. 1992. Quantitative determination of porphyrin in rat and human urine and evaluation of urinary porphyrin profiles during mercury and lead exposures. J. Lab. Clinic. Med. 120:272-281.

Fox, G.A., S.W. Kennedy, R.J. Norstrom and D.C. Wingfield. 1988. Porphyria in herring gulls: a biochemical response to chemical contamination of Great Lakes food chains. Environ. Toxicol. Chem. 7:831-839.

Kennedy, S.W. and G.A. Fox. 1990. Highly carboxylated porphyrin as a biomarker of polyhalogenated aromatic hydrocarbon exposure in wildlife: confirmation of the presence in Great Lake herring gull chicks in the early 1970's and important methodological details. Chemosphere 21:407-415.

Woods, J.S., M.A. Bowers and H.A. Davis. 1991. Urinary porphyrin profiles as biomarkers of trace metal exposure and toxicity: studies on urinary porphyrin excretion patterns in rats during prolonged exposure to methyl mercury. Toxicol. Appl. Pharmacol. 110:464-476.

Technique Name: Delta-Aminolevulinic Acid Dehydratase

Technique Type: Enzyme Inhibition
Matrix Type: Terrestrial and Aquatic Organisms
Ecosystem Level: Individual
Test Location: Laboratory

Description:

Delta-aminolevulinic acid dehydratase (ALAD) is an enzyme that catalyzes the formation of porphobilinogen, a precursor of heme. Often, ALAD is inhibited before other signs of toxicity become apparent. Field studies have revealed ALAD inhibition in fish, birds, and mammals exposed to various forms of lead. ALAD inhibition is best used as a indicator of exposure but not necessarily toxicity. ALAD inhibition can be measured in blood and/or liver samples.

Logistical Considerations:

Sample Collection:

Training: Minimal

Time: Minimal-MODERATE; Depends on the species.

Equipment: Dry ice is required to preserve field-collected samples.

Sample Analysis:

Training: MODERATE

Time: MODERATE

Equipment: A spectrophotometer is required.

Critique/Comments:

Assays of ALAD have the advantage of being relatively simple, inexpensive, accurate, and precise.

Key References:

Haux, C., A. Larsson, G. Lithner and M.L. Sjobeck. 1986. A field study of physiological effects on fish in lead-contaminated lakes. Environ. Toxicol. Chem. 5:283-288.

Hodson, P.V., B.R. Blunt and D.M. Whittle. 1984. Monitoring lead exposure of fish, In V.W. Cairns, P.V. Hodson and J.O. Nriagu, eds., Contaminant Effects on Fisheries. John Wiley & Sons, Toronto. pp 87-97.

Jakim, E. 1973. Influence of lead and other metals on fish δ-aminolevulinate dehydratase activity. J. Fish. Res. Bd. Canad. 30: 560-562.

Johansson-Sjobeck, M.-L. and A. Larsson. 1979. Effects of inorganic lead on delta amino-luvelinic acid dehydratase activity and haematological variables in the rainbow trout (Salmo gairdneri). Arch. Environ. Contam. Toxicol. 8: 419-431.

Scheuhammer, A.M. 1987. Erythrocyte α-aminolevulinic acid dehydratase in birds. I. The effects of lead and other metals in vitro. Toxicology 45:155-163.

Technique Name: Metabolic Products

Technique Type: Biochemical
Matrix Type: Biological Tissue
Ecosystem Level: Individual
Test Location: Laboratory

Description:

Many xenobiotic chemicals are converted to metabolic products in the organism soon after exposure. Detection of metabolites provides evidence of exposure to the xenobiotic chemical. Metabolites that can currently be used in environmental monitoring include metabolites of chlorinated hydrocarbons and PAHs, which can be detected in tissues, and metabolites of chlorinated phenols, resin acid metabolites, and PAHs, which can be detected in bile.

Logistical Considerations:

Sample Collection:

Training: MODERATE

Time: MODERATE

Equipment: Equipment depends on the metabolic product of interest. Liquid nitrogen and appropriate holding containers are needed for samples that may contain short-lived compounds while wet ice may be suitable for more stable compounds.

Sample Analysis:

Training: EXTENSIVE

Time: MODERATE

Equipment: Varies with the analyte of interest. Spectrophotmetry, fluorimetry, GC, HPLC, or some combination of these analytical techniques are very commonly applied methods for detection of toxicants in biological samples.

Critique/Comments:

This method provides direct evidence of exposure to compounds of interest.

Key References:

Krahn, M.M., M.S. Myers, D.G. Burrows and D.C. Malins. 1984. Determination of metabolites of xenobiotics in the bile of fish from polluted waterways. Xenobiotica 14:633-646.

Malins, D.C., B.B. McCain, D.W. Brown, S.-L. Chan, M.S. Myers, J.T. Landahl, P.G. Prohaska, A.J. Friedman, L.D. Rhodes, D.G. Burrows, W.D. Gronlund and H.O. Hodgins. 1984. Chemical pollutants in sediments and diseases of bottom-dwelling fish in Puget Sound, Washington. Environ. Sci. Technol. 18:705-713.

Melancon, M. J., R. Alscher, W. Benson, G. Kruzynski, R. F. Lee, H. C. Sikka and R. B. Spies. 1992. Metabolic products as biomarkers, In R. J. Huggett, R. A. Kimerle, P. M. Mehrle, Jr. and H. L. Bergman, eds., Biomarkers: Biochemical, Physiological, and Histological Markers of Anthropogenic Stress. Lewis Publishers, Boca Raton, FL, pp. 87-123.

Oikari, A. and T. Kunnamo-Ojala. 1987. Tracing of xenobiotic contamination of water with the aid of fish bile metabolites: a field study with caged rainbow trout (*Salmo gairdneri*). Aquat. Toxicol. 9:327-341.

Thakker, D.R., H. Yagi, W. Levin, A.W. Wood, A. H. Conney and D.M. Jerina. 1985. In M. W. Anders, ed., Bioactivation of Foreign Compounds. Academic Press, Orlando, FL, pp. 177-242.

Varansi, U., W.L. Reichert and J.E. Stein. 1989. ^{32}P-postlabeling analysis of DNA adducts in liver of wild English sole (*Parophrys vetulus*) and winter flounder (*Pseudopleuronectes americanus*). Cancer Res. 49:1171-1177.

Technique Name: Metallothionein Induction

Technique Type: Enzyme Induction
Matrix Type: Biological Tissue
Ecosystem Level: Individual
Test Location: Laboratory

Description:

Metallothioneins are low molecular weight, metal-binding proteins and oligonucleotides that are induced by exposure to a wide variety of heavy metals such as cadmium, copper, zinc, mercury, cobalt, nickel, bismuth, and silver. Metallothioneins can be used to assess exposure to heavy metals.

Logistical Considerations:

Sample Collection:

Training: Minimal

Time: MINIMAL-MODERATE; Depends on the species.

Equipment: Field collected samples must be frozen in liquid nitrogen.

Sample Analysis:

Training: EXTENSIVE

Time: MODERATE

Equipment: A centrifuge capable of 100,000 g is required.

Critique/Comments:

Considerable effort is required for calibration, using proteins of known molecular weights, of the described technique.

Data from studies using metallothioneins as biomarkers must be interpreted cautiously since many of these proteins can be induced by environmental stresses other than exposure to a toxicant.

Key References:

Benson, W.H., K.N. Baer and C.F. Watson. 1990. Metallothionein as a biomarker of environmental metal contamination: species-dependent effects, In J.F. McCarthy, L.R. Shugart, eds., Biomarkers of Environmental Contamination. Lewis Publishers, Boca Raton, Fl. pp 255-287.

Cope, W.G., J.G. Weiner, and G.J. Atchison. 1994. Hepatic cadmium, metal-binding proteins and bioaccumulation in bluegills exposed to aqueous cadmium. Environ

Hamilton, S.J. and P.M. Mehrle. 1986. Metallothionein in fish: review of its importance in assessing stress from metal contaminants. Trans Am. Fish. Soc. 115:596-609.

Langston, W.J. and M. Zhou. 1986. Evaluation of the significance of metal binding proteins in the gastropod *Littorina littorea*. Mar. Biol. 92:505-515.

Roch, M. and J.A. McCarter. 1984. Hepatic metallothionein in rainbow trout (*Salmo gairdneri*) as an indicator of metal pollution in the Campbell River System. Can J. Fish Aquat. Sci. 39:1596-1601.

Roesijadi, G. 1981. The significance of low molecular weight, metallothionein-like proteins in marine invertebrates: current status. Mar. Environ. Res. 4:167-179.

Technique Name: Stress Protein Induction

Technique Type: Enzyme Induction
Matrix Type: Biological Tisue
Ecosystem Level: Individual
Test Location: Laboratory

Description:

Certain classes of low molecular weight proteins are induced in response to a variety of environmental stressors including hyperthermia, sulfide-reactive agents, heavy metals, ethanol, glucose deprivation, viral infection, and anoxia. It has been suggested that these inducible proteins function to renature other proteins that have been denatured by exposure to an insulting agent. The presence of stress proteins can serve as an indication of exposure to an environmental stressor.

Logistical Considerations:

Sample Collection:

Training: MINIMAL

Time: MINIMAL-MODERATE; Depends on the species.

Equipment: Field collected samples must be preserved in liquid nitrogen until sample analysis.

Sample Analysis:

Training: EXTENSIVE

Time: MODERATE

Equipment: An ultracentrifuge is required. Equipment associated with the Western blotting technique is required.

Critique/Comments:

Although may stressor specific proteins have been induced and characterized in the laboratory, these proteins have not been widely utilized as a biomarker of environmental contaminant exposure.

Key References:

Moromoto, R., A. Tissieres and C. Georgopoulos, eds. 1990. The Role of the Stress Response in Biology and Disease. Cold Springs Harbor, New York: Cold Spring Harbor Laboratory.

Sanders, B.M. 1990. Stress Proteins: Potential as multitiered biomarkers, In L. Shugart, and J. McCarthy, eds., Environmental Biomarkers. Lewis Publishers. Inc., Chelsea, MI, pp. 165-191.

Technique Name: Clastogenicity Tests

Technique Type: DNA Modification
Matrix Type: Biological Tissues
Ecosystem Level: Individual
Test Location: Laboratory

Description:

This technique is used to examine chromosomal aberrations induced by exposure to contaminants. Cells are usually examined in the mitotic phase for alterations, rearrangements, breakage, and translocations. These effects have been correlated with the presence of mutagens and carcinogens.

Logistical Considerations:

Sample Collection:

Training: MINIMAL

Time: MINIMAL-MODERATE; Depends on the species.

Equipment: Samples collected in the field must be preserved in liquid nitrogen.

Sample Analysis:

Training: EXTENSIVE

Time: MODERATE

Equipment: Flow cytometer is required for accurate analysis.

Critique/Comments:

Cells must be in the process of dividing for this assay.

Key References:

McBee, K. J.W. Bickhman, K.W. Brown and K.C. Donnelly. 1987. Chromosomal aberrations in native small mammals (*Peromyscus leucopus* and *Sigmodon hispidus*) at a petrochemical waste disposal site: I. Standard karyology. Arch. Environ. Contam. Toxicol. 16:681-688.

McBee, K. and J.W. Bickham. 1989. Mammals as bioindicators of environmental toxicity, In H.H. Genoways, ed., Current Mammalogy. Plenum Press. New York, NY. pp. 37-88.

Pesch, G.G. and C.E. Pesch. 1980. *Neanthes arenaceodentata* (Polychaeta: Annelida): a proposed cytogenetic model for marine genetic toxicology. Can. J. Fish. Aquat. Mar. Genet. Toxicol. 37:1225-1228.

Thompson, R.A., G.D. Schroder, and T.H. Connor. 1988. Chromosomal aberrations in the cotton rat, *Sigmodon hispidus*, exposed to hazardous waste. Environ. Molec. Mutagen. 11:359-367.

Tice, R.R., B.G. Ormiston, R. Boucher, C.A. Luke and D.E. Paquette. 1987. Environmental biomonitoring with feral rodent species, In S.S. Sandhu, D.M. Demanine, M.J. Mass, M.M. Moore and J.L. Mumford, eds., Short-term Bioassays in the Analysis of Complex Environmental Mixtures, Vol. V. Plenum Press. New York, NY.

U.S. Environmental Protection Agency. 1985. Toxic Substances Control Act Test Guidelines: Final Rules. 40CFR, parts 796, 797, and 798.

Technique Name: Ames Test

Technique Type: DNA Modification
Matrix Type: Water or Extracts of Solid-Phase Materials
Ecosystem Level: Individual
Test Location: Laboratory

Description:

The Ames test can be used to assess the mutagenic potential of contaminants of a water sample. Test strains of histidine-dependent *Salmonella* bacteria are cultured on media containing nutrients, a microsomal preparation, and the potential mutagen. Standard test strains contain mutations that make them more susceptible to mutations than wild-type bacteria. A toxic response is measured by a decrease in the number of revertants, i.e. cells that are able to grow in the absence of histidine. The number of revertants is a measure of the ability of the mutagen to produce a change in DNA.

Logistical Considerations:

Sample Collection:

Training: MINIMAL

Time: MINIMAL

Equipment: A centrifuge may required for extraction of compounds from soil sediment or other matrices.

Sample Analysis:

Training: MINIMAL

Time: MODERATE

Equipment: Bacterial culturing facilities are required.

Critique/Comments:

Results should be interpreted cautiously with regard to extrapolation to carcinogenicity and to other species.

Key References:

Ames, B.N., J. McCann and E. Yamaski. 1975. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian mutagenicity test. Mutation Res. 31:347.

Maron, D.M. and B.N. Ames. 1983. Revised methods for the Salmonella mutagenicity test. Mutation Res 113:173.

Technique Name: Detection of DNA Adducts

Technique Type: DNA Modification
Matrix Type: Biological Tissues
Ecosystem Level: Individual
Test Location: Laboratory

Description:

Exogenous compounds or their metabolites may covalently bind to DNA. Shortly after exposure, organismal exposure to a contaminant can be ascertained by the detection of these adducts. Currently, techniques used to detect and quantify DNA adducts utilize P-postlabeling, HPLC/fluorescence, and immunological techniques. Tests currently under development utilize gas chromatography, gas chromatography/mass spectroscopy, capillary zone electrophoresis-mass spectroscopy, and fluorescence line-narrowing techniques.

Logistical Considerations:

Sample Collection:

Training: MINIMAL

Time: MINIMAL-MODERATE; Depends on the species.

Equipment: Samples collected in the field must be stored in liquid nitrogen.

Sample Analysis:

Training: MODERATE

Time: MINIMAL

Equipment: Several techniques can be employed to detect DNA adducts. Equipment needed differs with each of these procedures. A rapid and commonly employed technique requires a fluorimeter.

Critique/Comments:
Techniques currently used to detect DNA adducts are limited in sensitivity or specificity. The use of DNA adducts for use in assessment of exposure has recently begun to be validated in field studies using the P-postlabeling assay. Currently, the P-postlabeling technique is semi-quantitative, laborious, and moderate in cost.

Key References:

Dunn, B., J. Black and A. Maccubbin. 1990. ^{32}P-postlabeling analysis of aromatic DNA adducts in fish from polluted areas. Cancer Res. 47:6543-6548.

Halbrook, R.S., R. L. Kirkpatrick, D.R. Bevan and B. P. Dunn. 1992. DNA adducts detected in muskrats by ^{32}P-postlabeling analysis. Environ. Toxicol. Chem. 11:1605-1613.

Lower, W.R., F.A. Ireland, and B.M. Judy. 1991. ^{32}P-postlabeling for DNA adduct determination in plants, In J.W. Gorsuch, W.R. Lower, M.A. Lewis, and W. Wang, eds., Plants for Toxicity Assessment: Second Volume. American Society for Testing and Materials, Philadelphia, PA. pp 291-307.

Rahn, R., S. Chang, J.M. Holland and L.R. Shugart. 1982. A fluorometric-HPLC assay for quantitating the binding of benzo[a]pyrene metabolites to DNA. Biochem. Biophys. Res. Commun. 109:262-269.

Randerath, K., M. Reddy and R.C. Gupta. 1981. ^{32}P-postlabeling analysis for DNA damage. Proc. Natl. Acad. Sci. U.S.A. 78:6126-6129.

Santella, R.M., R. Gasparo and L. Hsieh. Quantitation of carcinogen-DNA adducts with monoclonal antibodies. Prog. Exp. Tumor Res. 31:63-75.

Technique Name: Secondary Modification of DNA

Technique Type: DNA Modification
Matrix Type: Biological Tissue
Ecosystem Level: Individual
Test Location: Laboratory

Description:

These tests are designed to detect modifications of DNA such as strand breakage, changes in minor base composition, or an increase in the level of unscheduled DNA synthesis. The alkaline unwinding assay is a sensitive technique used to detect strand breakage. Exogenous compounds can affect minor base composition by altering the activity of enzymes responsible for controlling the amount of methylated deoxyribonucleoside present in DNA. Alteration of these enzymes can result in hypomethylation of DNA, which can be detected by ion-exchange chromatography. Damaged DNA is repaired by unscheduled DNA synthesis. Detection of unscheduled DNA synthesis serves as a general indicator of genotoxic exposure.

Logistical Considerations:

Sample Collection:

Training: MINIMAL

Time: MINIMAL- MODERATE; Depends on the species.

Equipment: Samples collected in the field must be stored in liquid nitrogen.

Sample Analysis:

Training: EXTENSIVE

Time: MINIMAL

Equipment: Thin-layer chromatography is employed for this assay. A densitometer is required.

Critique/Comments:

Tests used to detect secondary modifications of DNA have not been fully developed for use as general biomarkers for environmental species. However, the strand breakage assay is currently being evaluated for environmental applications and has been tested using several environmental species, including oysters and mussels, desert rodents, and turtles. Strand breakage assay and minor nucleoside content assay measure a loss of DNA integrity but do not identify the chemical responsible.

Key References:

Shugart, L.R. 1988. An alkaline unwinding assay for the detection of DNA damage in aquatic organisms. Marine Environ. Res. 24:321-325.

Shugart, L.R. 1990. Biological monitoring: testing for genotoxicity, In J.F. McCarthy and L.R. Shugart, eds., Biological Markers of Environmental Contaminants. Lewis Publishers, Inc., Boca Raton, FL, pp 205-216.

Shugart, L.R. 1990. DNA damage as an indicator of pollutant-induced genotoxicity, In W.G. Landis and W.H. van der Schalie, eds., 13th Symposium on Aquatic Toxicology Risk Assessment. ASTM Publishers, Philadelphia, PA. pp. 205-216.

Shugart, L.R. 1990. 5-methyl deoxycytidine content of DNA from bluegill sunfish (*Lepomis macrochirus*) exposed to benzo[a]pyrene. Environ. Toxicol. Chem. 9:205-208.

Technique Name: Trace Metals in Tissues

Technique Type:	Assessment of Accumulation
Matrix Type:	Biological Tissues
Ecosystem Level:	Individual
Test Location:	Laboratory

Description:

Selected endpoints, typically comprised of biochemical or physiological responses, in individual organisms can be measured to provide sensitive indices of exposure or sublethal stress. Although biomarkers currently cannot be used to determine effects at population, community, or ecosystem levels, carefully selected biomarkers can serve as very sensitive monitoring tools to detect exposure, to assess sublethal stress, and to delineate zones of impact. When feasible, determination of tissue residues is recommended for assessment of exposure. The species chosen and the tissues selected for analysis will depend largely on the ecology of the site and information about contaminating metals. Since most metals bioaccumulate, concentrations in tissues can be measured directly by methods such as atomic absorption spectroscopy, inductively-coupled plasma, and neutron activation analysis. Methods differ in cost, sensitivity to various metals, and availability.

Logistical Considerations:

Sample Collection:

Training: MINIMAL

Time: MINIMAL - MODERATE depending on the species.

Equipment: Depends on the species.

Sample Analysis:

Training: MODERATE

Time: MINIMAL

Equipment: An atomic absorption spectrophotometer is required for analysis of most metals.

Critique/Comments:

Standard methods have been developed for the acquisition and analysis of biological samples for many metals of widespread environmental concern.

Analysis of metal concentrations biological tissue can provide direct evidence of exposure if levels are significantly higher in organisms from contaminated sites than in organisms collected from a control. Despite the usefulness of this technique, it is important to realize that detection of metals in tissue gives little indication of possible effects to the exposed organism.

This method has been widely used with bryophytes; lichens; and terrestrial and aquatic plants invertebrates, amphibians, reptiles, birds, and mammals. A large volume of literature exists, which addresses accumulation of most every heavy metal element.

Key References:

Hunter, B.A., and M.S. Johnson. 1982. Food chain relationships of copper and cadmium in contaminated grassland ecosystems. Oikos 38:108-117.

Jenkins, D.W. 1980. Nickel accumulation in aquatic biota, In J.O. Nriagu, ed., Nickel in the Environment. John Wiley and Sons, New York. pp. 283-338.

Johnson, M.S., R.D. Roberts, M. Hutton, M.J. Inskip. 1978. Distribution of lead, zinc, and cadmium in small mammals from polluted environments. Oikos 30:153-159.

Richardson, D.H.S., P.J. Beckett and E. Nieboer. 1980. Nickel in lichens, bryophytes, fungi, and algea, In J.O. Nriagu, ed., Nickel in the Environment. John Wiley and Sons, New York. pp. 367-406.

Timmermans, K.R. 1993. Accumulation and effects of trace metals in freshwater invertebrates, In R. Dallinger and P.S. Rainbow, eds., Ecotoxicology of Metals in Invertebrates. Lewis Publishers, Boca Raton, FL. pp 133-148.

Technique Name: Skeletal Abnormalities

Technique Type: Physiological -- Gross Indices
Matrix Type: Whole Body Evaluation
Ecosystem Level: Individual
Test Location: Field

Description:

Various chemical contaminants have been reported to cause skeletal and/or vertebral abnormalities. These chemicals include the heavy metals zinc, cadmium, and lead; the organochlorine compounds kepone, toxaphene, mirex, Aroclor 1254, 2,4-DMA, and chlordecone; the organophosphate pesticides parathion and malathion; trifluralin; and crude oil. Skeletal and vertebral abnormalities of environmental species have been used to monitor pollution effects. Several techniques can be used to assess skeletal abnormalities.

Logistical Considerations:

Sample Collection:

Training: MINIMAL

Time: Can be MINIMAL-EXTENSIVE depending on the elusive nature of the species to be examined and sample size requirements.

Equipment: Depends on the species to be collected.

Sample Analysis:

Training: MODERATE

Time: MINIMAL for individual samples.

Equipment: Typical laboratory equipment is required.

Critique/Comments:

Key References:

Bengtsson, B.E. 1979. Biological variables, especially skeletal deformities in our fish for monitoring marine pollution. Philos. Trans. R. Soc. London 286:457-464.

Bengtsson, A. and B.-E. Bengtsson. 1983. A method to registrate spinal and vertebral anomalies in fourhorn sculpin, *Myoxocephalus quadricornis* L. (Pisces). Aquilo Ser. Zool. 22:61-64.

Bengtsson, B.-E., A. Bengtsson and M. Himberg. 1985. Fish deformities and pollution in some Swedish waters. Ambio 14:32-35.

Goede, R. 1993. Fish health/condition assessment procedures. Part 1. Procedures manual. Utah Division of Wildlife Resources. Fisheries Exp. Sta. Logan, Utah. 31pp.

Goede, R. 1993. Fish health/condition assessment procedures. Part 2. A color atlas of autopsy classification categories. Utah Division of Wildlife Resources. Fisheries Exp. Sta. Logan, Utah. 3pp with 64 color plates.

Mayer, F.L., B.-E. Bengtsson, S.J. Hamilton and A. Bengtsson. 1988. Effects of pulp mill and ore smelter effluents on vertebrae of fourhorn sculpin: laboratory and field comparisons, In W.J. Adams, G.A. Chapman and W.G. Landis, eds., Aquatic Toxicology and Hazard Assessment, ASTM STP 971. American Society for Testing Materials, Philadelphia. pp 406-419.

Meyer, F.P. and L.A. Barclay. 1990. Field manual for the investigation of fish kills. U.S. Department of the Interior, Fish and Wildlife Service. Resource Pub. 177. Washington, DC. 120pp.

Technique Name: Hepatic Histopathology

Technique Type: Histopathological
Matrix Type: Biological Tissue
Ecosystem Level: Individual
Test Location: Laboratory

Description:

Several symptoms and forms of liver damage can serve as useful biomarkers of toxicant effects. Coagulative cellular necrosis is a currently useful indicator of toxicant exposure. Hyperplasia, which living cells undergo following necrosis, is another biomarker of exposure. Three types of hepatocytomegaly -- hepatocellular hypertrophy, megalocytosis, and hepatocytomegaly arising from swelling of perinuclear endoplasmic reticulum cisternae -- have been observed as responses to environmental contaminants in numerous fish species. Foci of cellular alteration form as an early stage of hepatic neoplasia. Detection of these foci have been used as a biomarker of exposure. The hepatic adenoma is an intermediate stage between cellular alteration and carcinoma. In addition to foci of cellular alteration, adenomas and hepatocellular carcinomas are present biomarkers. Cholangioma, cholangiocarcinoma, and mixed hepato-cholangiocellular carcinoma can all be used as biomarkers.

Logistical Considerations:

Sample Collection:

Training: MINIMAL

Time: MINIMAL to moderate depending on the species to be collected.

Equipment: Standard laboratory equipment.

Sample Analysis:

Training: EXTENSIVE

Time: MODERATE

Equipment: A microtome is needed to prepare samples prior to microscopic examination.

Critique/Comments:

For each of the biomarkers discussed, links between laboratory results and environmental relevance have been demonstrated.

Key References:

Baumann, P.C., J.C. Harshbarger and K.J. Hartmann. 1990. Relationship between liver tumors and age in brown bullhead populations from two Lake Erie tributaries. Sci Tot. Environ. 94:71-87.

Harshbarger, J.C. and J.B. Clark. 1990. Epizootiology of neoplasms in bony fish of North America. Sci. Total Environ. 94:1-32.

Hendricks, J.D., T.R. Meyers and D.W. Shelton. 1984. Histological Progression of hepatic neoplasia in rainbow trout *Salmo gairdneri*. Natl. Cancer Inst. Monogr. 65:321-336.

Hinton, D.E. and D.J. Lauren. 1990. Integrative histopathological approaches for detecting effects of environmental stressors of fishes, In S.M. Adams, ed., Biological Indicators of Fish Community Stress, Amer. Fish. Soc. Special Pub.

Kent, M.L., M.S. Myers, D.E. Hinton, W.D. Eaton and R.A. Elston. 1988. Suspected toxicopathic hepatic necrosis and megalocytosis in pen-reared Atlantic salmon *Salmo salar* in Puget Sound, Washington, U.S.A. Dis. Aquat. Org. 49:91-100.

Meyers, T.R. and J.D. Hendricks. 1985. Histopathology, In G.M. Rand and S.R. Petrocelli, eds., Fundamentals of Aquatic Toxicology. Hemisphere Publishing Corp., Washington, D.C., pp. 283-331.

Technique Name: Macrophage Phagocytosis

Technique Type: Immunological
Matrix Type: Biological Tissue
Ecosystem Level: Individual
Test Location: Laboratory

Description:

Macrophage phagocytosis activity is assessed by adding blood cells from environmental organisms to tissue culture medium. The culture is incubated for approximately 24 to 48h. Either fluorescent yeast cells or fluorescent latex particles are then added to the cultures. Phagocytosis activity is measured by counting the number of fluorescent particles macrophages in the culture ingest.

Logistical Considerations:

Sample Collection:

Training: MINIMAL

Time: MINIMAL to MODERATE depending on the species to be sampled.

Equipment: Depends on the species.

Sample Analysis:

Training: MODERATE

Time: MINIMAL

Equipment: Incubator, Cell Harvester, Sterile Hood, Pipettors, Autoclave, Centrifuge, Coulter Counter, Incubator, Fluorescent Microscope.

Critique/Comments:

Use of any immune dysfunction or suppression assays should be viewed as a component of an integrated risk analysis. As the immune system is closely integrated with many organ systems and functions, and is a network capable of rapid cell proliferation and differentiation, it is susceptible to effects from contaminant exposure. Immune dysfunction or suppression is

a good measure of exposure over time and may reflect the results of simultaneous exposures to contaminants. Further, the ability of the immune system to rapidly proliferate memory cells makes it more suitable for chronic or repeated short exposures to contaminants than for assessing single, acute exposures. However, these tests are currently not capable of identifying the specific compounds responsible for inducing the effects, and no single change in an immune function has been shown to be pathognomic for a specific compound or class of chemicals. There appears to be considerable species-related variation which standardization of assays should help to minimize. Testing for immune dysfunction is appropriate for screening (Tier I) testing, but identification of the mechanisms causing the dysfunction will generally require Tier II testing.

Blood samples taken from individuals must be used the same day as collection.

Key References:

Exon, J.H., L.D. Koller, P.A. Talcott, C.A. O'Reilly and G.J. Henningsen. 1986. Immunotoxicity testing: an economical multiple-assay approach. Fund. Appl. Toxicol. 7:387-397.

Luster, M.I., A.E. Munson, P.T. Thomas, M.P. Holsapple, J.D. Fenders, K.L. White, Jr., L.D. Lauer, D.R. Germolec, G.J. Rosenthal and J.H. Dean. 1988. Development of a testing battery to assess chemical-induced immunotoxicity: National Toxicology Program's guidelines for immunotoxicity evaluation in mice. Fund. Appl. Toxicol. 10:2-19.

McBee, K. and J.W. Bickham. 1988. Peetrochemical-related DNA damage in wild rodents detected by flow cytometry. Bull. Environ. Contam. Toxicol. 40:343-349.

Weeks, B.A., R.J. Huggett,J.E. Warinner and E.S. Matthews. Macrophage responses of estuarine fish as bioindicators of toxic contamination, In J.F. McCarthy and L.R. Shugart, eds., Biomarkers of Environmental Contamination, Boca Raton, FL, pp. 193-201.

Zeeman, M.G. and W.A. Brindley. 1981. Effects of toxic agents on fish immune systems: a review, In R.P. Sharma, ed., Immunologic Considerations in Toxicology, Vol. II. CRC Press, Inc., Boca Raton, FL.

Zellikoff, J.T., N.A. Enane, D. Gowser, K.S. Squibb and K. Frenkel. 1991. Development of fish peritoneal macrophages as a model for higher vertebrates in immunotoxicological studies. Fund. Appl. Toxicol. 16:576-589.

Technique Name: Lymphocyte Blastogenesis

Technique Type: Immunological
Matrix Type: Biological Tissue
Ecosystem Level: Individual
Test Location: Laboratory

Description:

Macrophage phagocytosis activity is assessed by adding a sample of whole blood from environmental organisms to tissue culture medium containing mitogen. Cells are radiactively labeled by adding ^{3}H-thymidine to the culture. Cells are then harvested and the blastogenic response measured by comparing the mean disintegrations per minute in mitogen-stimulated wells with that of unstimulated wells.

Logistical Considerations:

Sample Collection:

Training: MINIMAL

Time: MINIMAL to MODERATE depending on the species to be sampled.

Equipment: Depends on the species.

Sample Analysis:

Training: MODERATE

Time: MINIMAL

Equipment: Incubator, Cell Harvester, Sterile Hood, Scintillation Counter, Pipettors, Autoclave.

Critique/Comments:

Blood samples taken from individuals must be used the same day as collection.

Key References:

Redig, P.T., J.L. Dunnette and V. Sivanandan. 1984. Use of whole blood lymphocyte stimulation test for immunocompetency studiesn in bald eagles, red-tailed hawks, and great horned owls. Am. J. Vet. Res., 45:2342-2346.

Rocke, T.E., T.M. Yuill and R.D. Hinsdill. 1984. Oil and related toxicant effects on mallard immune defenses. Environ. Res. 33:343-352.

Sharma, R.P. and R.V. Reddy. 1983. Toxic effects of chemicals on the immune system, In Immunotoxicology. Academic Press, New York, N.Y. pp. 555-591.

Technique Name: Microbial Toxicity Tests

Technique Type: Biochemical
Matrix Type: Leachate, surface water, sediments
Ecosystem Level: Individual
Test Location: Laboratory

Description:

Short-term microbial tests are based on inhibition of the activities of bacteria, algae, and fungi. These tests are versatile and cost-effective tools. Microbial toxicity tests include adenosine triphosphate (ATP) assays, enzymatic activity assays, bioluminescence assays, and microbial growth tests. By measuring ATP synthesis, effects of a toxicant can be gauged by comparing density of a treated bacterial colony with that in a control colony after several generations of bacterial cell growth. Enzymatic inhibition by toxicants could be an underlying cause of toxicity to cells. Toxicity tests, therefore, have been developed to assess inhibition of biosynthesis of enzymes, inhibition of enzyme function, and genetic interference, which leads to the loss of proper enzyme functioning. Bioluminescence assays are based on inhibition of the cellular electron transport system within the marine bacterium *Photobacterium phosphoreum*. Microbial growth tests assess population growth or cell motility.

Logistical Considerations:

Sample Collection:

Training: MINIMAL

Time: MINIMAL

Equipment: See references.

Sample Analysis:

Training: MINIMAL

Time: MINIMAL

Equipment: Water extraction techniques require a centrifuge capable of revolution speeds greater than 5000 rpm. Solvent extraction techniques require freeze-drying. Equipment needed may be different for other extraction techniques. A colorimeter is needed for most enzymatic activity tests. Many standardized tests require analytical instruments used specifically for microbial toxicity testing.

Critique/Comments:

These tests can readily be used to assess a wide range of toxicants in water, soil, sediments, sewage effluents, and leachates either directly or after concentration and/or extraction of water and organic solvents. Sensitivity of the test organism to a toxicant can vary with the type of test and toxicant. Many microbial toxicity tests have been standardized and are commercially available through various sources.

Key References:

ASTM. 1994. Annual book of ASTM standards. Water and Environmental Technology. Volume 11:04. Pesticides; resouce recovery; hazardous substances and oil spill responses; waste management; biological effects. American Society for Testing and Materials. Philadelphia, PA. 1619 pp.

American Public Health Association (APHA). 1985. Standard Methods for the Examination of Water and Wastewater. 17th ed. American Public Health Association, Washington, DC.

Bitton, G. and B. Koopman. 1986. Biochemical tests for toxicity screening, In G. Bitton and B.J. Dutka, eds., Toxicity Testing Using Microorganisms, Vol. 1, CRC Press, Boca Raton, FL, pp.27-55.

Bulich, A.A. 1986. Bioluminescent assays, In G.Bitton and B.J. Dutka, eds., Toxicity Testing Using Microorganisms, Vol.1. CRC Press, Boca Raton, FL, pp 57-74.

Carter, M.R. (ed.). 1993. Soil Sampling and Methods of Analysis. Lewis Publishers, Inc. Boca Raton, FL. 864 pp.

Holme-Hansen, O. 1973. Determination of total microbial biomass by measurements of 90 adenosine triphosphate, In L.H. Stevenson and R.R. Lowell, eds., Estuarine Microbial Ecology. University of South Carolina Press, Columbia, SC.

Xu, H. and B.J. Dutka. 1987. ATP-TOX system: A new rapid sensitive bacterial toxicity screening system based on the determination of ATP. Toxicity Assess. 2:149-166.

Technique Name: Microbial Ecological Effects Tests

Technique Type: Biochemical
Matrix Type: Soil and surface water
Ecosystem Level: Population/Community
Test Location: Laboratory

Description:

Processes that contribute to the cycling of carbon, nitrogen, sulfur, and phosphorus are among the most ecologically significant processes that contribute to the well-being of ecosystems. Certain processes such as nitrification and sulfur oxidation are mediated exclusively by specific groups of microorganisms whose activity can be assessed by their rates of metabolic processes. The cycling of the four elements listed above are especially valuable in environmental assessment. Assays for two of these, nitrogen and sulfur, have been developed. Nitrogen-transformation assays are conducted by adding various concentrations of a water sample or an extract from contaminated soil to a nitrifying soil microbial culture. The effects of a toxicant on sulfur transformations are assessed by adding dilutions of contaminated water or soil extracts to a culture that is actively mineralizing sulfur. Mineralization rates are determined by recovery of the $^{35}SO_4^{2-}$ isotope of Sulfoquinovose.

Logistical Considerations:

Sample Collection:

Training: MINIMAL

Time: MINIMAL

Equipment: MINIMAL

Sample Analysis:

Training: MODERATE

Time: MODERATE

Equipment: Sulfur-transformation assays require the use of scintillation counting equipment.

Critique/Comments:

Key References:

Klute, A., ed. 1965. Methods of Soil Analysis. Part 1: Physical and Mineralogical Methods. Am. Soc. Agronomy, Madison, WI.

Lees, H. and J.H. Quastel. 1946. Biochemistry of nitrification in soil. I. Kinetics of, and the effect of poisons on, soil nitrification, as studied by a soil perfusion technique. Biochem. J. 40:803-814.

Strickland, T.C. and J.W. Fitzgerald. 1983. Mineralization of sulfur in sulfoquinovose by forest soils. Soil Biol. Biochem. 15:347-349.

Technique Name: Microtox™

Technique Type: Luminescence Bioassay
Matrix Type: Water, extract (water or solvent) for aqueous-phase test; soil and sediment for solid-phase test.
Ecosystem Level: Organismal
Test Location: Laboratory

Description:

Although aqueous-phase testing with Microtox™ has been readily available for many years, solid-phase testing has only recently been commercially available. Both aqueous-phase and soil-phase tests with Microtox™ directly measure biological activity in water (or soil and sediment-derived extracts), and sediment or soil, respectively. Both test systems use luminescent bacteria (*Photobacterium phosphoreum*) to measure the biological effects on culture metabolism) that may be associated with exposure. Altered cellular metabolism may affect the intensity of light output from the organism. When these changes in light output are expressed, estimates of biological effects may be derived from screening or concentration-response curves that yield EC_{50}s (concentration of sample associated with a 50% reduction in light intensity) from plotted data. Unlike the aqueous-phase test where the sample (i.e., surface water, groundwater, sediment pore water or soil eluate) is directly tested (samples may be filtered), the solid-phase test requires a pre-testing extraction step. During the extraction, a "micro-eluate" is prepared from a soil sample (ca 0.3 gram), then incubated at 15°C. Following incubation, the soil-diluent slurry is filtered, and the filtrate is subsequently analyzed using the Microtox™ analyzer.

Logistical Considerations:

Sample Collection:

Training: MINIMAL.

Time: MINIMAL.

Equipment: A Microtox™ analyzer is required.

Sample Analysis:

Training: MINIMAL.

Time: MINIMAL.

Equipment: A Microtox™ analyzer is required.

Critique/Comments:

The results of several studies of pure compounds and complex chemical mixtures suggests that aqueous-phase testing with Microtox™ generally agrees with standard fish and invertebrate toxicity tests. Solid-phase testing with Microtox™, however, does not have a comparable data base established to compare with standard soil tests (e.g., earthworms survival). Furthermore, the solid-phase test does not take into account bacteria adsorbed to soil particles. Aqueous-phase testing and solid-phase testing with Microtox™ should both be performed in conjunction with other assessment methods, for example, animal or plant tests as previously noted (Warren-Hicks, et al., 1989). As a direct measure of altered soil structure and function, and for interpretation of ecological effects, the solid-phase Microtox™ currently requires the support of adequately defined site-specific reference soils, as well as a comparative data base that relates solid-phase Microtox™ test results with soil "health". These tests are performed with a marine bacterium; therefore, testing with soil eluates should be compared to standard soils toxicity tests to determine relevance to the soil type being tested.

Key References:

Bulich, A.A. 1986. Bioluminescent assays. Pages 57-74. In: G. Bitton and B.J. Dutka, eds. Toxicity Testing Using Microorganisms, Vol. 1. CRC Press, Boca Raton, FL.

Curtis, C., A. Lima, S.J. Lorano, and G.D. Veith. 1982. Evaluation of a bacterial bioluminescence bioassay as a method for predicting acute toxicity of organic chemicals to fish. Pages 170-178. In: J.G. Pearson, R.B. Foster, and W.E. Bishop, eds. Aquatic Toxicity and Hazard Assessment, STP 766, American Society for Testing and Materials. Philadelphia, PA.

Microbics Corporation. 1992. Microtox™ manual. Microbics Corporation, Carlsbad, CA.

Munkittrick, K.R., E.A. Power, and G.A. Sergy. 1991. The relative sensitivity of Microtox™, daphnid, rainbow trout, and fathead minnow acute lethality tests. Environ. Toxicol. and Water Quality. 6:35-62.

Warren-Hicks, W., B. Parkhurst, and S. Baker, Jr. (eds). 1989. Ecological assessment of hazardous waste sites. EPA/600/3-89/013. U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, OR.

Technique Name: Soil-core Microcosm

Technique Type: Soil Microcosm
Matrix Type: Soil
Ecosystem Level: Community, Organismal
Test Location: Field, and Greenhouse or Environmental Chamber

Description:

The soil-core microcosm test potentially measure the adverse effects, or toxicity, of chemicals in either defined or complex chemical mixture exposures. Originally, the 60-cm deep by 17-cm diameter terrestrial soil-core microcosm was designed to yield chemical effects data in soils collected from grassland or agricultural systems, but the method may be adapted for other soil types as necessary. The cylinder containing the intact soil core is collected from a site using stainless steel extraction tubes; laboratory testing is completed on the intact core. Routine physicochemical analyses are completed on the soil, e.g., percent organic material, cation exchange capacity, and nutrient analysis, and in conjunction with field surveys, vegetation and soil biota are characterized. Once in the laboratory, the soil core can be manipulated following a site-specific sampling and analysis plan, but ideally exposure conditions occur in a greenhouse or environmental chamber. The ASTM E1191 (1991) standard guide outlines numerous exposure methods. Potential endpoints measured in a soil-core microcosm study are numerous, but ecological endpoints that are routinely considered include productivity measurements, and measurements of plant health, nutrient loss and chemical fate testing. Recently, the method has been adapted to evaluate fate and effects of hazardous waste chemicals used in military training (Checkai, et. al., 1993).

Logistical Considerations:

Sample Collection:

Training: MINIMAL to collect, set-up and maintain soil cores.

Time: MODERATE (several weeks to several months).

Equipment: A greenhouse or environmental chamber is required.

Sample Analysis:

Training: MINIMAL to MODERATE. A good understanding of soil chemistry is required.

Time: MINIMAL to MODERATE.

Equipment: A wet chemistry laboratory and analytical equipment (i.e., HPLC or GC) is required.

Critique/Comments:

The soil-core microcosm test has been standardized through ASTM E1191 (1991), and has been validated within an ecological risk assessment context for various chemical and biological hazards. The test is designed to evaluate the environmental fate, ecological effects, and environmental transport of chemicals, both liquid and solid, and genetically-engineered microbial agents that may be released to terrestrial systems. For chemicals, the methods can be used to evaluate toxicity or adverse effects on growth and reproduction of native vegetation or crops and the uptake and cycling of nutrients in a soil/plant system. Although soil-core microcosm has been used in various hazard and risk assessment settings, no regulatory precedence exits for routinely testing site soils using the soil-core microcosm. Within applied contexts, the method has proven useful to evaluations of complex chemical waste, hazardous wastes, and agricultural chemicals. The soil-core microcosm potentially yields data that will be directly relevant to any soil contamination evaluation, and its limitations are those inherent to microcosms and laboratory tests in general. However, if reference soils are available for concurrent testing, the soil-core microcosm test can yield information that could be significant to an ecological effects assessment for contaminated soils.

Key References:

ASTM E1191. 1991. Standard guide for conducting a terrestrial soil-core microcosm test. Annual book of ASTM standards. Volume 11.04. Pesticides; Resource Recovery; Hazardous Substances and Oil Spill Responses; Waste Disposal; Biological Effects. American Society for Testing and Materials (ASTM). Philadelphia, PA 19103.

Checkai, R.T., R.S. Wentsel, C.T. Phillips, and R.L. Yon, 1993. Controlled environment soil-core microcosm unit for investigating fate, migration, and transformation of chemicals in soils. J. Soil Contam. 2(3):229-243.

Van Voris, P., D. Tolle, M.F. Arthur, J. Chesson, and T.C. Zwick. 1984. Development and validation of terrestrial microcosm test system for assessing ecological effects of utility wastes. EPRI Publication N. EA-3672, Final Project Report. Electric Power Research Institute, Palo Alto, CA.

Van Voris, P., D. Tolle, M.F. Arthur, and J. Chesson. 1985. Terrestrial microcosms: validation, applications, and cost-benefit analysis. In Multi-species toxicity testing, Pergamon Press, New York, NY. pp. 117-142.

Van Voris, P., D. Tolle, and M.F. Arthur. 1985. Experimental terrestrial soil-core

microcosm test protocol. A method for measuring the potential ecological effects, fate, and transport of chemicals in terrestrial ecosystems. 600/3-85/047, PNL-5450. Environmental Research Laboratory, Corvallis, OR.

Technique Name: Soil Microbial Activity

Technique Type: Biochemical Bioassay
Matrix Type: Soil
Ecosystem Level: Biochemical
Test Location: Laboratory

Description:

Activity rates, as determined by enzyme studies, nucleic acid production and incorporation into biomass or nucleic acids, can be used as indices of total soil microbiological activity. Measurement of microbial activity usually involves addition of a substrate for a particular enzyme to utilize. Incubation times should be kept as short as possible to prevent microbial growth and reproduction. Sorption of the substrate or products on the surfaces of soil and clay particles needs to be prevented, limited, or measured. Measurement of substrate disappearance, enzyme presence, or product appearance must be kept as simple as possible, and usually is determined by a color change in the medium (disappearance of substrate or appearance of product changes pH and a pH sensitive dye is present in the medium), by change in turbidity, or by the production of a precipitate or chemical whose presence can be assayed by spectrophotometry. Methods available include: dehydrogenase assay, ATP content and adenylate energy charge (AEC), incorporation of radiolabelled nucleic acids, and calorimetry (heat production). Activity is measured as a change in color (spectroscopy) or by calorimetry.

Logistical Considerations:

Sample Collection:

Training: MINIMAL.

Time: MINIMAL.

Equipment: MINIMAL.

Sample Analysis:

Training: MINIMAL to record data, MINIMAL to MODERATE to evaluate data.

Time: MINIMAL.

Equipment: A calorimeter or spectrometer is required.

Critique/Comments:

A much more extensive database is needed. Major factors whose effects must be considered in establishing a baseline for interpretation of these measurements are: 1) localization of enzymes, cells, substrates and nucleic acids in soil, 2) standardization of methodology, 3) sorption of substrates, products and cells by soil clay and organic fractions, 4) nutrient cycling during long incubation assays, and 5) sampling of field soils and incubation in the laboratory gives potential rates and not *in situ* rates. General enzymes are produced by a wide variety of microorganisms, requiring the toxicant to affect a general reduction in the activity of soil heterotrophs before a reduction in enzyme activity is evident. Therefore, toxicants with limited or targeted biological activity, e.g., non-heavy metal pollutants, will rarely show a general effect. The positive aspects of assaying enzyme activity are the well established, rapidly performed, inexpensive procedures, which can be performed on whole soils, as well as soil extracts. These tests should be performed after standard toxicity tests to delineate specific toxic effects. Either a suite of enzyme assays must be performed, or some knowledge of impact must be available in order to choose one or two indicator enzyme assays.

Key References:

Christensen, G.M., D. Olson, and B. Reidel. 1982. Chemical effects on the activity of eight enzymes: A review and a discussion relevant to environmental monitoring. Environ. Res. 29:247-255.

Dutka, B.J. and G. Bitton, eds. 1986. Toxicity Testing Using Microorganisms, Vol. 2. CRC press, Boca Raton, FL.

Dutton, R.J., G. Bitton, and B. Koopman. 1988. Enzyme biosynthesis versus enzyme activity as a basis for microbial toxicity testing. Toxicity Assess. 3:245-253.

Eiland, F. 1985. Determination of adenosine triphosphate (ATP) and adenylate energy charge (AEC) in soil and use of adenine nucleotides as measures of soil microbial biomass and activity. Danish. J. Plant Soil Sci. S:1777:1-193.

Ladd, J.N. 1985. Soil Enzymes. IN D. Vaughan and R.E. Malcom (eds). Soil organic matter and biological activity. pp. 175-221. Martinus Nijhoff, Dordrecht, The Netherlands.

Lenhard, G. 1968. A standardized procedure for the determination of dehydrogenase activity in samples from anaerobic treatment systems. Wat. Res. 2:161-167.

Nannipieri, P., S. Grego, and B. Ceccanti. 1990. Ecological significance of the biological activity in soil. Soil Biochemistry 6:293-355.

Nannipieri, P., C. Ciardi, L. Badalucco, and S. Casella. 1986. A method to determine DNA and RNA. Soil Biol. Biochem. 18:275-281.

Orgenics Ltd. 1985a. The Toxi-chromotest, Version 2 (US). Orgenics Ltd., P.O. Box 360, Yavne 70650, Israel.

Orgenics Ltd. 1985b. The SOS Chromotest Blue Kit, TwoStep Version 3. Orgenics Ltd.,

P.O. Box 360, Yavne 70650, Israel.
Sparling, G.P. 1981. Heat output of the soil biomass. Soil Biol. Biochem. 13:373-376.
Tyler, G. 1974. Heavy metal pollution and soil enzymatic activity. Plant Soil 41:303-311.

Technique Name: Soil Lipid Chemistry

Technique Type: Soil Biochemistry
Matrix Type: Soil
Ecosystem Level: Biochemical
Test Location: Laboratory

Description:

The chemical composition of soil lipids is the direct result of the nature and reactivity of the various compounds added to soil from plant litter, animals, insects and microorganisms. Analysis of soil lipid chemistry can be used to assess bacterial and fungal community composition shifts and quantify essential soil characteristics. Two approaches hold significant promise with respect to soil lipids. First, the identity of soil organism groups can be determined using lipid signatures of particular groups, such as families, genera and species. Lipid-structure signatures from particular microbial groups can indicate subtle shifts in the composition of affected soils. Second, past biodegradation processes, hydrophobic properties, reactivity, and soil development can be assessed by analyzing soil lipids. To develop the lipid signature library, soil is spread on plates, colonies which grow on the plate are chosen based on morphology, the organism grown in liquid cultures, tested for purity and a portion of that culture extracted for lipids. These lipids must then be analyzed for the specific signature compounds. Test soils are then extracted for lipids, the extracts analyzed, and compared to known lipid signatures. Recent advances in cross polarization magic angle spin nuclear magnetic resonance (NMR) and mass spectrometry have opened new horizons for the characterization of soil lipids, such that different types of carbon (aliphatic C, protein branching patterns, long alkyl chains, carbohydrates, OH-substituted aliphatics, aromatics, phenolic and carboxyl C) can be distinguished.

Logistical Considerations:

Sample Collection:

Training: MODERATE to EXTENSIVE to extract lipids from soil.

Time: MODERATE to extract lipids.

Equipment: MODERATE to extract lipids.

Sample Analysis:

Training: MODERATE to EXTENSIVE to operate analytical equipment.

Time: MINIMAL to MODERATE.

Equipment: EXPENSIVE, a mass spectrometer or magnetic resonance imaging equipment is required.

Critique/Comments:

Soil lipid biochemistry techniques have demonstrated a high correlation of soil lipids with heavy metal contamination. Changes in lipid chemistry have been used to assess changes in microbial diversity and changes in fungal distribution patterns in impacted soils. In order to incorporate lipid biochemistry into a reliable technique for ecological risk assessment, methods development is required including: better techniques and equipment to extract and characterize chemically highly complex (especially for organisms) and polymerized (especially for soils) lipids; improved knowledge about mechanisms of inhibitory action of certain lipids on microbial populations and seed germination; assessment of biodegradability of various types of lipids in cultivated and uncultivated soil; and evaluation of the effect of certain lipids on soil structure. Reliable extraction efficiency of lipids from the sample, whether soil or organisms in soil, remains a problem. Characterization of lipids is time-consuming and, if new structures occur, difficult. Effects of different soil communities on lipid expression by individual organisms is a completely unknown interaction at this time.

Key References:

Dinel, H., M. Schnitzer, and G.R. Mehuys. 1990. Soil lipids: Origin, nature, content, decomposition and effect on soil physical properties. Soil Biochem. 6:397-429.

Nordgren, A.E., E. Baath, and B. Soderstrom. 1985. Soil microflora in an area polluted by heavy metals. Can. J. Bot. 63:448-455.

Nordgren, A.E., E. Baath, and B. Soderstrom. 1983. Microfungi and microbial activity along a heavy metal gradient. Appl. Environ. Microbiol. 45:1829-1837.

Vestal, J.R. and D.C. White. 1989. Lipid analysis in microbial ecology. Quantitative approaches to the study of microbial communities. Bioscience 39:535-541.

Technique Name: Nitrogen Cycling in Soil

Technique Type: Soil Chemical Bioassay
Matrix Type: Soil
Ecosystem Level: Biochemical
Test Location: Laboratory

Description:

One very simple method is to collect soil and assess nitrogen pools (ammonium, nitrate, nitrite) at time zero and after incubation in plastic "zip-lock" bags. The difference indicates the potential nitrogen cycling rate. A second method is to add N-15 labeled ammonium to the soil and determine the rate at which it appears as nitrate-nitrite. For nitrification rates, one approach is to compare nitrification rates in contaminated soils to rates in uncontaminated soils. An alternative method is to add the soil to be tested to a sensitive culture of nitrifying bacteria and test for continued function of the bacterial culture. An aqueous suspension of a toxic substance is added to a culture of *Nitrosomonas europaea*. The conversion of ammonium to nitrite is quantified. Although there are no reports to our knowledge of this approach being used to assess toxicity in soil samples, the results of Powell and Prosser (1986) suggest that the method has potential usefulness. The rate of ammonium conversion to nitrate and/or nitrite, and the rate at which nitrate is converted to nitrate are the endpoints measured. The concentrations of ammonium, nitrate and nitrite are determined colorimetrically, using either autoanalyzers or laboratory spectrophotometers.

Logistical Considerations:

Sample Collection:

Training: MINIMAL.

Time: MINIMAL.

Equipment: MINIMAL.

Sample Analysis:

Training: MINIMAL.

Time: MINIMAL.

Equipment: Equipment is required to measure different oxidative states of nitrogen compounds.

Critique/Comments:

Of the major nitrogen transformations mediated by microorganisms, nitrogen cycling is one of the most important and directly related to plant productivity. In addition, nitrification of ammonium to nitrite and then to nitrate appears to be the most sensitive transformation to a wide range of potential toxicants. A broad database of information has been published on nitrification and the effects of various toxic chemicals. A literature search is needed to summarize this information and improve the interpretation of hazardous chemical impacts in these organisms and this process. In general, biogeochemical transformation of nitrate has been shown to be highly sensitive to pesticides, herbicides, and heavy metals. The nitrifying organisms used in these tests were cultures from standard sources. No attempt was made to seek strains isolated from nonpolluted waters or soils that may be particularly sensitive to toxicants. Because nitrification is known to be sensitive to a wide range of toxicants, it should be relatively easy to select for strains that are particularly sensitive to different groups of toxicants. Knowing the composition of toxicants at a given site, technicians could select one or more strains that are known to be particularly sensitive to the toxicants present on the site. The resulting tests should be very sensitive, rapid, and easy to perform by relatively untrained personnel.

Key References:

Domsch, K.H. 1970. Effects of fungicides on microbial populations in soil. In: Pesticides in Soil Ecology; Degradation and Movement Symposium. E. Lansing State University, Mich.

Parr, J.F. 1974. Effects of pesticides on microorganisms in soil and water. In: Pesticides in Soil and Water. Guenzi, W.D., J.L. Ahlrichs, M.E. Bloodworth, G. Chesters, and R.G. Nash (eds). Soil Sci. Soc. Amer. Inc. Madison. pp. 315-340.

Powell, S.J. and J.I. Prosser. 1986. Effect of copper on inhibition by nitrapyrin of growth of *Nitrosomonas europaea.*

Sato, C., S.W. Leung, and J.L. Schnoor. 1988. Toxic response of *Nitrosomonas europaea* to copper in inorganic medium and wastewater. Water Res. 22:1117-1127.

Tu. C.M. 1970. Effect of four organophosphorus insecticides on microbial activities in soil. Appl. Microbiol. 19:479-484.

Wainwright, M. 1978. A review of the effects of pesticides on microbial activity in soils. J. Soil Sci. 29:287-298.

Technique Name: Uptake and Utilization of Organic Compounds by Microbes

Technique Type: Uptake and Utilization of Chemicals
Matrix Type: Soils
Ecosystem Level: Biochemical
Test Location: Laboratory

Description:

Substrate uptake by bacteria and fungi can be used as a means of demonstrating toxic effects by following the fate of radiolabelled toxicant when added to a soil sample. This approach is most appropriate with organic toxicants that are broken down by organisms with relatively specialized function. Hydrocarbons, sugars or other substrates of interest are added to the soil. Their utilization is assayed by determining labeled CO_2 production, labeled biomass production, or disappearance of the labeled compound. Radiolabelled hydrocarbon uptake and incorporation into biomass have been used to demonstrate increased numbers of organisms capable of degrading crude oil and petroleum products in areas contaminated by those and related compounds. Thus, soils contaminated with degradable organics could be assayed for effects on the ability to utilize particular compounds by adding a particular radiolabelled compound and following it's fate. Additionally, assaying for the enrichment of organisms capable of using the toxicant in the impacted soil as compared to a standard soil, analogous to assaying for resistant microorganisms, could be performed.

Logistical Considerations:

Sample Collection:

Training: The laboratory must be licensed to use radiolabelled materials.

Time: MODERATE to EXTENSIVE to determine extent of degradation/utilization, and to determine persistence of the organisms.

Equipment: EXPENSIVE radiolabelled compounds are required.

Sample Analysis:

Training: MODERATE to learn analyses.

Time: MINIMAL.

Equipment: A scintillation counter is needed.

Critique/Comments:

Before this approach can be highly useful, we need to know how long after a pollutant enters the soil before enrichment of resistant, or degradatory organisms will occur, and how long the resistant/degradatory organisms persist in the environment after a pollutant has been degraded. This approach is beneficial for the remediation of impacted soil however. The organisms capable of degrading the pollutant can be isolated, high numbers grown in the laboratory, and used to inoculate the soil at the site. Since the organism was originally from the site, novel organisms are not being placed on-site. The organisms should be able to grow in the condition at the site, since they were originally isolated from the area. Testing is needed to make certain no changes in genetic capability of the organism occurs in laboratory culture. The main methodological drawbacks of this method are the need for relatively expensive radiolabelled isotopes, disposal of the radiolabelled test material, the specialized equipment needed for determining radiolabelled-compound degradation (a liquid scintillation counter) and the fact that no general-activity radiolabelled material is available. If a spectrum of effects is suspected, each substrate must be tested separately.

Key References:

Atlas, R.M. 1991. Microbial hydrocarbon degradation - bioremediation of oil spills. J. Chem. Tech. 52:149-156.

Dobbins, D.C., C.M. Aelion, and F. Pfaender. 1992. Subsurface, terrestrial microbial ecology and biodegradation of organic chemicals: A review. In Critical Reviews in Environmental Control. 22(1/2):67-136.

McCormick, N.G., J.H. Cornell, and A.M. Kaplan. 1981. Biodegradation of hexahydro-1,3,5-trinitro-1,3,5-trianyene. Appl. Environ. Microbiology. 42:817-823.

Nannipieri, P., S. Grego, and B. Ceccanti. 1990. Ecological significance of the biological activity in soil. Soil Biochem. 6:293-355.

Technique Name: **Soil Respiration**

Technique Type: Chemical Assay
Matrix Type: Soil Microorganisms
Ecosystem Level: Biochemical
Test Location: Laboratory, Field

Description:

Soil respiration is a general indicator of microbial activity, easily measured with relatively simple tools using easy-to-follow protocols. One main advantage is that respiration can be determined non-destructively on intact soils. The same volume of soil in the same plot of ground can be followed over time, a distinct advantage when trying to assess recovery in a system. A standardized air-tight container is placed over a known volume of soil, either in the field, or in laboratory pots. After 1 to 24 hours, the accumulation of carbon dioxide in the collecting vessel is determined and compared to controls. Respired gases can be collected by trapping in alkali (KOH), by removing a known volume of gas from the headspace of the chamber and analyzing for CO_2 with a gas chromatograph or respirometer.

Logistical Considerations:

Sample Collection:

Training: MINIMAL.

Time: MINIMAL.

Equipment: A gas chromatograph or respirometer is required.

Sample Analysis:

Training: MINIMAL.

Time: MINIMAL.

Equipment: A gas chromatograph or respirometer is required.

Critique/Comments:

Considerable data are available on the effects of toxic chemicals on respiration rates, although this information needs to be compiled into one source. In general, pesticides and heavy metals have significant impacts on respiration (Nohrstedt, 1987). In work with heavy metal contamination, respiration has been shown to be a useful measure of impact most likely because heavy metals have such broad effects on organisms. The most important soil characteristic influencing the toxic response was the clay content for Cd, Fe content for Cu, Pb, and Zn toxicity, and pH for Ni toxicity. Inhibition was the greatest in sand and lowest in the clay soils.

As with soil enzymes, all the organisms in soil contribute to soil respiration rates, including roots. Chemicals may impact only one component part of all the organisms present in soil and the impact on total respiration may be small compared to the respiration of all organisms present. Pinpointing the impacted organism is not possible with this method. Thus, the toxicant must have broad effects in order to disrupt all soil organism components or there is little likelihood that an effect will be seen. This metric should be used as a general indicator of serious and far-reaching impact of soil contaminants.

Key References:

Doelman, P. and L. Haanstra. 1984. Short-term and long-term effects of cadmium chromium, cooper, nickel, lead, and zinc on soil microbial respiration in relation to abiotic soil factors. Plant Soil 79:317-337.

Dumontet, S. and S.P. Mathur. 1989. Evaluation of respiration-based methods for measuring microbial biomass in metal-contaminated acidic mineral and organic acids. Soil Biol. Biochem. 21:431-435.

Grossbard, F. and H.A. Davies. 1976. Specific microbial responses to herbicides. Weed Res. 16:163-169.

Nohrstedt, H.O. 1987. A field study on forest floor respiration response to artificial heavy metal contaminated acid rain. Scand. J. For. Res. 2:13-19.

Parr, J.F. 1974. Effects of pesticides on microorganisms in soil water. In: Pesticides in Soil and Water. Guenzi, W.E., J.L. Ahlrichs, M.E. Bloodworth, G. Chesters, and R.G. Nash (eds). Soil Sci. Soc. Amer. Inc. Madison. pp. 315-340.

Technique Name: **Seed Germination and Root Elongation**

Technique Type: Germination and Growth
Matrix Type: Vascular Plant Seed and Seedling
Ecosystem Level: Individual
Test Location: Growth Chamber, Greenhouse

Description:

Seed germination tests require exposure of size-graded seeds (e.g., Lactuca sativa (lettuce)) to a chemical in a soil slurry adjusted to pH 6-10. Screening tests should be completed on uncut, homogenized soil samples. For definitive tests, EC_{50} estimates require at least three replicates of at least five test soil concentrations. After planting, 16-mesh cover sand is poured over each plate, the petri dishes subsequently placed into plastic bags, sealed, and incubated at $24\pm2°C$ for 120 hours in an environmental chamber. The first 48 hours of incubation occurs in complete darkness, and the last 72 hours occurs under 16:8 light:dark cycle. The endpoint for screening tests is percent germination. If definitive tests are completed, median effective estimates (EC_{50}s) may be calculated.

Root elongation evaluations estimate the adverse biological effects of soil eluates to lettuce seedlings (Lactuca sativa) in a 120-hour test. Screening evaluations may be completed using uncut soil eluates; if definitive tests follow, at least three replicates must be included as part of the test design. Root lengths are measured from the transition point between the hypocotyl and root to the end of the root tip. Root elongation results in screening tests are reported as percent reduction in root lengths in treatments relative to controls; in definitive tests, EC_{50}s (the concentration which inhibits root elongation by 50% relative to controls) may be calculated. For both tests, three replicates of negative and positive controls are required for definitive tests.

Logistical Considerations:

Sample Collection:

Training: MINIMAL.

Time: MINIMAL (120-hr).

Equipment: MODERATE. A refrigerator, pH meter, and supplemental lighting are required.

Sample Analysis:

Training: MINIMAL.

Time: MINIMAL.

Equipment: MINIMAL.

Critique/Comments:

Seed germination tests should be considered when field surveys suggest that plant communities have been impacted at a site, or when future land use may require a phytotoxicity evaluation as part of the soil contamination testing. The root elongation test measures biological activity of water soluble soil constituents, both contaminant and non-contaminant. The soil-derived eluate may be directly relevant to evaluations of soil contamination and groundwater quality relationships, or to evaluations of altered quality of surface water runoff from a contaminated site. Also, when soil contamination directly or indirectly impacts the plant rhizosphere, soil-derived eluates may provide information regarding interstitial water quality that potentially influences plants inhabiting contaminated soil. Both tests have been standardized and approved by USEPA and FDA.

The current data collection is heavily skewed toward north-temperate, agricultural species, particularly grasses and legumes; little information is available regarding less commercially important native plants and woody species.

Key References:

AOSA (Association of Official Seed Analysts). 1990. Rules for testing seeds. J. Seed Tech. 12:1-122.

CFR (Code of Federal Regulations). 1985. Rules and regulations; Section 797.2750, Seed germination/root elongation toxicity test. September 27, 1985. CFR 50 (188):39389-39391.

FDA (Food and Drug Administration). 1987. Sections 4.06 (Seed germination and root elongation); 4.07 (Seedling growth). In Environmental Assessment Technical Assistance Handbook. NTIS, PB 87-175345. U.S. Food and Drug Administration, Washington, D.C.

Linder, G., J.C. Greene, H. Ratsch, J. Nwosu, S. Smith, and D. Wilborn. 1990. Seed germination and root elongation toxicity tests in hazardous waste site evaluation: methods development and applications. In Plants for Toxicity Assessment, ASTM STP 1091. W. Wang, J.W. Gorsuch, and W.R. Lower, Eds., American Society for Testing and Materials. Philadelphia, PA. Pp. 177-187.

Ratsch, H. 1983. Interlaboratory root elongation testing of toxic substances on selected plant species. NTIS, PB 83-226. U.S. Environmental Protection Agency, Environmental Protection Agency, Washington, D.C.

Technique Name: Early Seedling Survival and Vegetative Vigor

Technique Type: Survival and Growth
Matrix Type: Vascular Plant
Ecosystem Level: Organismal
Test Location: Growth Chamber or Greenhouse

Description:

Seedlings are grown in soils collected on-site from identified sampling locations or are grown in soils dosed with known levels of contaminants. Plants identified for testing should be selected to meet the site-specific data needs (e.g., commercial seeds or native seeds), and should be grown under greenhouse or environmental chamber conditions specified by their species requirements. Supplemental lighting may be required to ensure sufficient photosynthetically active radiation under specified lighting regimens. Growth conditions, e.g., temperature and humidity, should be recorded daily as well as any additional exposure conditions that are critical to successful completion of the test. At test termination (usually 14 days), plant leaves and roots should be collected from each exposure and control replicate, and total biomass should be recorded as an endpoint for assessing plant vigor. Supplemental endpoints may also be defined during the problem formulation phase of an ecological effects study design (e.g., physiological and morphological indicators of plant health). In order to adequately interpret test endpoints, soil samples should be split after being prepared for testing and submitted for physicochemical characterization (e.g., soil moisture and pH, textural analysis, total nitrogen and total organic matter, and cation exchange capacity).

Logistical Considerations:

Sample Collection:

Training: MINIMAL.

Time: MODERATE (14-day).

Equipment: MODERATE to EXPENSIVE, a growth chamber or greenhouse is required.

Sample Analysis:

Training: MINIMAL.

Time: MINIMAL.

Equipment: MINIMAL to MODERATE. A pH meter is required.

Critique/Comments:

Vegetative vigor and early seedling survival tests are designed to extend the information gathered using short-duration phytotoxicity tests. For example, seed germination tests and root elongation tests may not adequately show chronic effects that are potentially associated with low concentration, environmental contaminant exposures. Vegetative vigor and early seedling survival tests may also be designed to address site-specific questions related to contaminant uptake into plant tissues, if chemical analytical data are collected concurrent with harvest data.

Within a regulatory setting, various agencies have outlined the requirements and specifications for vegetative vigor and early seedling survival tests (Holst and Ellwanger 1982; OECD 1984; FDA 1987). As with short-term phytotoxicity tests, the comparative data base is sparse, and testing with north-temperate, agricultural species, particularly grasses and legumes is emphasized. Little information is available for less commercially important native plants and woody species.

Key References:

CFR (Code of Federal Regulations). 1985. Rules and regulations; Section 797.2800, Early seedling growth toxicity test. September 27, 1985 CFR 50 (188):39391-39393.

FDA (Food and Drug Administration). 1987. Sections 4.06 (Seed germination and root elongation); 4.07 (Seedling growth). In Environmental Assessment Technical Assistance Handbook. NTIS, PB 87-175345. U.S. Food and Drug Administration, Washington, D.C.

Gorsuch, J.W., R.O. Kringle, and K.A. Robillard. 1990. Chemical effects on the germination and early growth of terrestrial plants. In Plants for Toxicity Assessment, ASTM STP 1091. W. Wang, J.W. Gorsuch, and W.R. Lower, eds., American Society for Testing and Materials. Philadelphia, PA. Pp. 49-58.

OECD (Organization for Economic Co-Operation and Development). 1984. OECD guidelines for testing of chemicals. Director of Information, OECD. 2, rue Andre Pascal, 75775 Paris Cedex 16, France.

Technique Name: **Rooted Aquatic and Wetland Plants**

Technique Type: Growth and Physiology
Matrix Type: Vascular Plants (Aquatic)
Ecosystem Level: Organismal
Test Location: Growth Chamber, Greenhouse, Test Ponds

Description:

Standardized plant toxicity tests have been developed for species native to freshwater/estuarine and wetland environments. Aquatic species commonly tested include Hydrilla verticillata and Potamogeton pectinatus (sago pondweed). In the Hydrilla v. test, screening tests or definitive tests using soil or sediment dilutions may be completed with the standardized test system. Regardless of screening or definitive test application, each sediment or hydric soil sample should be evaluated in triplicate with three plants per jar following a 14-day exposure. Hoagland's nutrient is added to the jars and incubation occurs in an environmental chamber under controlled temperature (25±1°C) with continuous cool white fluorescent light (40 uE $m^{-2}sec^{-2}$). Test endpoints may include estimates of shoot and root growth, as well as biological markers indicative of sublethal contaminant effects (Byl and Klaine 1991). P. pectinatus tests require a four-week test period.

Testing wetland sites includes the marsh plant, Echinochloa crusgalli. After 14-day exposure under controlled environmental conditions, plants are counted and weighed to measure survival and growth endpoints. For interpretation of ecological effects, methods are outlined for designing reference sediments or wetland soils, if natural reference materials are not available (Walsh, et al 1990).

Logistical Considerations:

Sample Collection:

Training: MINIMAL to MODERATE.

Time: MODERATE (14-30 days).

Equipment: MINIMAL to MODERATE to regulate environmental factors.

Sample Analysis:

Training: MINIMAL.

Time: MINIMAL.

Equipment: MINIMAL.

Critique/Comments:

Plant tests with rooted aquatic plants have had previous application in evaluating contaminated sediments, and have a relatively well established toxicity data base in the literature for selected contaminants. Testing with marsh plants has only recently been fully developed, and the data base for hazardous waste site applications is limited. Technically, these tests, whether using aquatic rooted plants, or freshwater or estuarine wetland plants, are relatively straight forward, yet ecologically relevant contaminant information can be gained in a relatively short time period. Although the number of test species is relatively limited, the increasing awareness regarding the ecological significance of wetland habitats should support a consideration of site-specific laboratory testing.

Key References:

Byl, T.D. and S.J. Klaine. 1991. Peroxidase activity as an indicator of sublethal stress in the aquatic plant Hydrilla verticillata (Royle). In Plants for Toxicity Assessment: Second Volume. ASTM STP 1115, J.W. Gorsuch, W.R. Lower, W. Wang, and M.A. Lewis, eds., American Society for Testing and Materials. Philadelphia, PA. Pp. 101-106.

Fleming, W.J., M.S. Ailstock, J.J. Momot, and C.M. Norman. 1991. Response of sago pondweed, a submerged aquatic macrophyte, to herbicides in three laboratory culture systems. In Plants for Toxicity Assessment: Second Volume. ASTM STP 1115, J.W. Gorsuch, W.R. Lower, W. Wang, and M.A. Lewis, eds., American Society for Testing and Materials. Philadelphia, PA. Pp. 267-275.

Walsh, G.E., D.E. Weber, L.K. Brashers, and T.L. Simon. 1990. Artificial sediments for use in tests with wetland plants. Environ. Exper. Botany 30:391-396.

Walsh, G.E., D.E. Weber, T.L Simon, and L.K. Brashers. 1991. Toxicity tests of effluents with marsh plants in water and sediment. In Plants for Toxicity Assessment: Second Volume. ASTM STP 1115, J.W. Gorsuch, W.R. Lower, W. Wang, and M.A. Lewis, eds., American Society for Testing and Materials. Philadelphia, PA. Pp. 517-525.

Technique Name: **Plant Uptake Bioassay**

Technique Type: Contaminant uptake in plants
Matrix Type: Vascular Plant (nutsedge)
Ecosystem Level: Organismal
Test Location: Laboratory

Description:

This bioassay is appropriate for estimating mobility of contaminants into the environment through plant uptake in wetland and marsh environments as well as drier upland sites. The test method was originally designed by the US Army Corps of Engineers Waterways Experiment Station (WES) to evaluate field-collected dredge materials. Field-collected sediment or wetland soils are physicochemically characterized, mixed and placed in pots. Cyperus esculentus (nutsedge) is planted in the contaminated sediment/soil and maintained under flooded and/or upland conditions for 45 days in a greenhouse or environmental chamber under controlled conditions (32$\pm$2°C daylight temperatures, 21$\pm$2°C night temperatures under 1,200 $\mu E/m^2$ photosynthetically active radiation (PAR) and > 50% relative humidity). Above-ground biomass is measured and contaminant content of leaves is determined.

Logistical Considerations:

Sample Collection:

Training: MINIMAL to germinate and maintain plants.

Time: MODERATE (45-day test).

Equipment: MINIMAL.

Sample Analysis:

Training: MINIMAL to measure biomass. MODERATE to extract and analyze for heavy metals and organic compounds.

Time: MINIMAL to MODERATE to extract and analyze contaminants.

Equipment: MODERATE to EXPENSIVE for equipment to measure contaminant concentrations.

Critique/Comments:

This method offers a technique for measuring uptake of a contaminant in a plant with a relatively short life-cycle. However, to date, the technique has only been applied to uptake of trinitrotoluene (TNT) in dredged material. Yellow nutsedge testing, although well-defined and applied in the U.S. Army Corps of Engineers dredge materials program, is unexploited for ecological assessments. Within an ecological risk assessment, various remedial options such as sediment dredging may suggest that testing with Cyperus esculentus be incorporated into site management plans. Also, the method could be adapted for soils.

Few technical support laboratories are currently providing tests with yellow nutsedge. Furthermore, equipment needed for extraction, purification, and analysis of contaminants in plant tissue is relatively expensive. Site-specific interpretation of ecological effects associated with potential responses in the test would center upon "laboratory to field" extrapolation error and interspecies variability with respect to contaminant-mediated adverse effects.

Key References:

Folsom, Jr., B.L. and R.A. Price. 1991. A plant bioassay for assessing plant uptake of contaminants from freshwater soils or dredged material. In Plants for toxicity Assessment: Second Volume. ASTM STP 1115, J.W. Gorsuch, W.R. Lower, W. Wang, and M.A. Lewis, eds., American Society for Testing and Materials. Philadelphia, PA. Pp. 172-177.

WES (U.S. Army Corps of Engineers Waterways Experiment Station). 1989. A plant bioassay for assessing plant uptake of heavy metals from contaminated freshwater dredged material. Technical Note EEDP-04-11. U.S. Army Corps of Engineers Waterways Experiment Station, Vicksburg, MS.

Technique Name: **TOXSCREEN**

Technique Type: Whole Plant Toxicity
Matrix Type: Vascular Plant (mature)
Ecosystem Level: Organismal
Test Location: Greenhouse or Environmental Growth Chamber

Description:

TOXSCREEN tests whole plant (non-seedling) response to hydroponically-applied chemicals. Plants, e.g. soybean (Glycine max) and barley (Hordeum vulgare) are grown in hydroponic culture in an environmentally-controlled greenhouse or growth chamber for 28 days, then exposed to chemicals in solution for 3-5 days. Test conditions are maintained under constant photoperiod (16/8 light/dark, light intensity of 350 $umolm^{-2}s^{-1}$ at top of canopy) at 25/21±2°C and 50-70% relative humidity. When appropriate, solvent systems may be used as carriers, for example, when rhizosphere exposures are designed to reflect site-specific conditions. Soil eluates may also be used. Toxicity endpoints routinely include survival and growth, although exposure systems could be designed that allow additional measurements for estimating sublethal effects (McFarlane, et al. 1990).

Logistical Considerations:

Sample Collection:

Training: MINIMAL to set up and maintain hydroponics system.

Time: MODERATE to construct and calibrate hydroponics system. MINIMAL to perform experiments (3-5 days).

Equipment: MODERATE to EXPENSIVE. Greenhouse or growth chambers required.

Sample Analysis:

Training: MINIMAL to test for growth endpoints, MINIMAL to MODERATE to test for physiological responses.

Time: MINIMAL to measure most endpoints.

Equipment: MINIMAL for growth endpoints. MINIMAL to EXPENSIVE to measure physiological responses.

Critique/Comments:

TOXSCREEN should be considered primarily as a screening test, particularly if contaminants of concern are water soluble and conducive to hydroponic exposures. Depending upon site-specific characteristics, target analytes could be used in single compound or defined chemical mixture exposures, and if sufficient soil were collected, eluates could be used as the exposure medium. An advantage of the test over previous screening methods is that whole plants are used rather than seedlings. One disadvantage is that soils cannot be tested directly. TOXSCREEN was originally designed with regulatory applications being the central focus and therefore should be considered in developing sampling and analysis plans, depending upon site-specific contingencies. Few technical support laboratories are currently providing tests with these organisms; owing to its recent description, TOXSCREEN is not commercially available. If adequate facilities and technical support are available, the test exposure is relatively short; however, adequate technical considerations must be made to assure that plant materials are available for testing (e.g., hydroponic nursery facility or commercial sources).

Technique Name: **Vascular Plant Life-cycle Tests**

Technique Type: Plant Bioassay
Matrix Type: Vascular Plant
Ecosystem Level: Organismal
Test Location: Greenhouse or growth chamber

Description:

Two hydroponic test systems using short life-cycle plants are potentially applicable to ecological effects assessments for hazardous waste sites. Water-soluble constituents of waste site chemical mixtures may be evaluated with either Arabidopsis thaliana or Brassica rapa. Exposures occur in double-pot, static-replacement systems where a vermiculite-filled growth container is nested above a second larger pot that serves as a nutrient solution reservoir. Nutrients and water move from the nutrient reservoir to the vermiculite via polyester wicks that are draped between the two pots. Seeds are uniformly planted on the surface of the vermiculite, and greenhouse conditions or large growth chambers assure similar growing conditions for all plants. Depending upon the exposure period and growth conditions, plants will set seeds and mature. Exposure periods (approximate seed-to-seed life-cycle) are 28-36 days for A. thaliana and 36-44 days for Brassica rapa. Endpoints include total biomass, individual organ biomass (e.g., stems, leaves, roots, seeds, fruits), leaf and flower structure, and initial flowering date.

Logistical Considerations:

Sample Collection:

Training: MINIMAL.

Time: MODERATE, 28-44 days.

Equipment: MODERATE to EXPENSIVE, a hydroponic system and greenhouse or growth chamber are required.

Sample Analysis:

Training: MINIMAL.

Time: MINIMAL.

Equipment: An analytical balance is required for biomass endpoints.

Critique/Comments:

Both of these full life-cycle tests are intended to address toxicity endpoints that are inadequately considered in standardized plant tests measuring seed germination and root elongation. Exposures are hydroponic, and contaminant water solubility may limit exposures for some chemicals. If eluates are used in regard to soil contaminant as sources for potential groundwater and rhizosphere contamination, a direct measure of "worst case" can be addressed using these systems. Relatively large volumes of eluate may be required for these hydroponic systems relative to that volume used in the standard root elongation test, however; defined chemical mixtures similar to those found in site-soils could be incorporated into the test system's nutrient solution and used as an alternative exposure system. Few technical support laboratories are currently providing tests with these organisms; establishing this system in one's own facility may prove to be too costly and time-consuming. A book has recently been published describing detailed methods in Arabidopsis research (Koncy et al, 1992).

Key References:

Koncy, C., N. Chua, and J. Schell. 1992. Methods in Arabidopsis Research. World Scientific, River Edge, NJ.

Ratsch, H.C., D.J. Johndro, and J.C. Mc Farlane. 1986. Growth inhibition and morphological effects of several chemicals in Arabidopsis thaliana (L.) Heynh. Environ. Contam. Toxicol. 5:55-60.

Shimabuku, R.A., H.C. Ratsch, C.M. Wise, J.U. Nwosu, and L.A. Kapustka. 1991. A new plant life-cycle bioassay for assessment of the effects of toxic chemicals using rapid cycling Brassica. In Plants for Toxicity Assessment: Second Volume, ASTM.

STP 1115. J.W. Gorsuch, W.R. Lower, W. Wang, and M.A. Lewis, eds. American Society for Testing and Materials, Philadelphia, PA. pp. 365-375.

Technique Name: **Plant Tissue Culture Tests**

Technique Type: Plant Bioassay
Matrix Type: Plant Cell and Callus Tissue
Ecosystem Level: Organismal
Test Location: Laboratory

Description:

For evaluating subacute effects, particularly chemical-related alterations in plant metabolism, plant cell and tissue culture techniques have become well developed over the past ten years. Suspension cultures of commercially important plant species e.g., soybean (Glycine max) and wheat (Triticum aestivum) are exposed to contaminants added to the culture nutrient medium. Exposures pertinent to an ecological effects assessment require that eluates be prepared from site-soil. The indirect effects of soil contaminants could then be evaluated by supplementing the test nutrient medium with eluate spikes. Alternatively, if the contaminant history for the site was reliable, or if analytical information regarding soil contaminants was available, defined chemical mixtures could be added as supplements to the nutrient medium. Endpoints include biomass, metabolic fate and biotransformation of chemicals. Similar test methods have been developed using callus cultures.

Logistical Considerations:

Sample Collection:

Training: MODERATE to EXTENSIVE to learn tissue culture techniques.

Time: MODERATE (30-60 days) to grow cultures.

Equipment: MODERATE, tissue culture media and materials, and environmentally controlled chambers or rooms are required.

Sample Analysis:

Training: MODERATE to EXTENSIVE training in plant biochemistry is required to assess metabolic end products.

Time: MODERATE to purify and quantitate metabolites.

Equipment: MODERATE to EXPENSIVE analytical equipment is required to quantitate metabolites.

Critique/Comments:

Both callus and cell suspension cultures of various plant species have been used in evaluating subacute chemical effects in plants, primarily by addressing the metabolic fate of xenobiotics (e.g., herbicides) in plants; but little correlative work has been completed to address the ecological interpretation of these in vitro plant cell and tissue culture methods. The method(s) outlined and summarized here are relatively early in the standardization process, and are not intended to be "stand alone" tests. Rather, the strengths of these method(s) lie in their contribution to evaluating phytotoxicity in species which are difficult to assess with whole plant tests. Metabolic effects of chemicals, ascertained by tissue-culture analysis, may be used to explain site-specific effects associated with soil exposures, e.g., diminished vigor in woody shrubs or poor reproductive performance in forbes, found during field surveys.

Tissue culture and analytical methods are costly and time-consuming. Few technical support laboratories provide tissue-culture techniques and chemical exposure/analyses. The cost-effectiveness of using tissue-culture techniques should be considered before implementing these methods in a risk assessment.

Key References:

Ebing, W., A. Haque, I. Schuphan, H. Harms, C. Langebartels, D. Scheel, K.T. von der Trenck, and H. Sanderman. 1984. Ecochemical assessment of environmental chemicals: draft guideline of the test procedure to evaluate metabolism and degradation of chemicals by plant cell cultures. Chemosphere 13:947-957.

Harms, H. and C. Langebartels. 1986. Standardized plant cell suspension test systems for an ecotoxicologic evaluation of the metabolic fate of xenobiotics. Plant Sci. 45:157-165.

Wickloff, C. and J.S. Fletcher. 1991. Tissue culture as a method for evaluating the biotransformation of xenobiotics by plants. In Plants for Toxicity Assessment: Second Volume, ASTM STP 1115. J.W. Gorsuch, W.R. Lower, W. Wang, and M.A. Lewis, eds. American Society for Testing and Materials, Philadelphia, PA. pp. 250-257.

Zilkah, S. and J. Gressel. 1977b. Cell cultures vs. whole plants for measuring phytotoxicity. III. Correlations between phytotoxicities in cell suspension cultures, calli, and seedlings. Plant & Cell Physiol. 18:815-820.

Technique Name: **Plant Community Structure Mesocosm**

Technique Type: Community Structure Analysis of Vascular Plants
Matrix Type: Vascular Plant
Ecosystem Level: Community
Test Location: Greenhouse or Field

Description:

A mesocosm, representative of a larger system, is used to determine the impacts of the release of chemicals from hazardous waste sites on plant community structure. Seed for testing is obtained commercially from regional native seed supply sources, and the plant community to be tested can be defined. Alternatively, a seed bank is collected from a reference location and used in evaluations of site-soil. If seed bank sources are used, past land and chemical use should be documented and any confounding effects owing to the selection of reference area seed bank should be acknowledged. Raised beds are typically used as exposure containers and may be located in the field or in the greenhouse, depending upon the site-specific study design. Defined seed mixtures or seed bank are then incorporated into the soil, and depending upon the study design, irrigation and fertilization can be specified. Each site-specific study plan may differ in their details for analysis of plant community responses to contaminated soils, but for waste sites with similar contaminant histories and similar habitat settings, study designs may be nearly identical. Exposures will vary with respect to duration depending upon regional characteristics (e.g., native plant species composition when initiating test from seed bank). Target plant species may be identified for specific focus in the study. Or, ecological endpoints may be identified for analyzing community-level responses. For example, percent vegetative cover, total biomass, species diversity and richness may be determined. The level of analytical detail should be determined initially in the study design. Regardless of the study design, identification of a reference soil is critical in the evaluation of soil contamination and its effects on native plants.

Logistical Considerations:

Sample Collection:

Training: MINIMAL to construct and maintain mesocosm.

Time: EXTENSIVE (3-9 months) to establish a plant community within the mesocosm.

Equipment: A greenhouse may be required.

Sample Analysis:

Training: MINIMAL to MODERATE.

Time: MODERATE to EXTENSIVE for measuring community-level responses.

Equipment: MINIMAL.

Critique/Comments:

Intended use: Although concentration-response relationships may be designed as part of the vegetation evaluation completed with a plant community study, the method may be more valuable as a screening method complementary to controlled plant test, e.g., vegetative vigor and early seedling survival. By using both an "ecotoxicity test" to measure plant community responses to contaminated soil and an organismic-level test like vegetative vigor and early seedling survival, uncertainty in the risk characterization for the site may be more adequately addressed on the basis of site-specific empirical information. As a field test, or greenhouse test, the plant mesocosm exposure beds are relatively easy to establish, but the test is time and labor intensive, owing to the real-time growth required for biomass measurements and data collection and reduction for evaluating community structure.

Key References:

Pfleeger, T. 1991. Impact of airborne pesticides on natural plant communities. In Plant tier testing: a workshop to evaluate nontarget plant testing in Subdivision J Pesticide Guidelines. 600/9-91/041. U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, OR.

Weinstein, L.H. and J.A. Laurence. 1989. Indigenous and cultivated plants as bioindicators. In Biologic markers of air-pollution stress and damage in forests. Committee on Biologic Markers of Air-Pollution Damage in Trees, G.M. Woodwell (Chair). National Research Council. National Academy Press. Washington, D.C. pp. 195-204.

Weinstein, L.H., J.A. Laurence, R.H. Mandl, and K. Walti. 1990. Use of native and cultivated plants as bioindicators and biomonitors of pollution damage. In W. Wang, J.W. Gorsuch, and W.R. Lower (eds.). Plants for Toxicity Assessment. ASTM STP 1091. American Society for Testing and Materials, Philadelphia, PA. pp. 117-126.

Technique Name: **Phytotoxicity Testing with Ambient Air Exposure Systems**

Technique Type: Visual Injury, Physiological Analysis
Matrix Type: Vascular Plant (terrestrial)
Ecosystem Level: Organismal, Community
Test Location: Field, Greenhouse, Growth Chamber

Description:

Field, greenhouse, and growth chamber exposure systems, originally designed to examine the effects of gaseous air pollutants and acidic deposition on vegetation can be adapted for ecological risk assessments. Known concentrations of pollutant gases (e.g., O_3, SO_2, volatile organics) and/or wet deposition contaminants (e.g., acidic precipitation) have been applied to crop plants, forest trees, and native vegetation using these systems. Additionally, field systems can be used to regulate pollutant exposure on-site by selectively filtering contaminants. Measurement endpoints include foliar injury, biomass growth, physiological measurements (e.g., photosynthesis, respiration) and bioaccumulation of contaminants in plant tissues.

Recently, a field method was developed to test the impact of smokes/obscurants used by the U.S. Army in training exercises on native vegetation (Sadusky, et. al., 1993, Skelly, 1990). Open-top field exposure chambers were adapted to expose tree seedlings to hexachloroethane smoke. Following four exposures at two-week intervals, particulate deposition was estimated and visual injury was quantified. This method may be adapted for use with other air pollutants found at military installations.

Logistical Considerations:

Sample Collection:

Training: MINIMAL to set up and maintain systems.

Time: MODERATE (7-30 days) to study acute effects, EXTENSIVE (2 months-2 years) to study chronic effects.

Equipment: EXPENSIVE to install and maintain exposure systems.

Sample Analysis:

Training: MINIMAL for most endpoint measurements.

Time: MINIMAL.

Equipment: MINIMAL to MODERATE. Analytical equipment maybe necessary for measurement of physiological endpoints.

Critique/Comments:

The method(s) outlined and summarized here are well developed but have not been standardized. The strengths of these method(s) lie in their potential contribution to evaluating exposure pathways that generally have not been considered within an ecological effects assessment (e.g., exposures to military smokes/obscurants). In determining whether ambient air exposures are critical to the ecological effects assessment, various elements influencing exposure should be considered. In general these elements may be categorized as: contaminant physicochemical attributes in the atmosphere; soil, habitat, and atmospheric conditions that may influence exposure and non-exposure periods; and biological attributes of receptors - plant or animal - that may be exposed via ambient air pathways. These systems are very expensive to establish and maintain and technical support laboratories with established systems may not be equipped to adapt their systems to tests with military chemicals.

Key References:

Heagle, A.S., D.E. Body, and W.W. Heck. 1973. An open-top field chamber to assess the impact of air pollution on plants. J. Environ. Qual. 2:365-368.

Hogsett, W.E., D. Olszyk, D.P. Ormord, G.E. Taylor, Jr., and D.T. Tingey. 1987. Air pollution exposure systems and experimental protocols. Volume 1: A review and evaluation of performance. 600/3-87/037a. U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, OR.

Hogsett, W.E., D. Olszyk, D.P. Ormord, G.E. Taylor, Jr., and D.T. Tingey. 1987. Air pollution exposure systems and experimental protocols. Volume 2: Description of facilities. 600/3-87/037b. U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, OR.

Sadusky, M.C., J.M. Skelly, M. Simini, R.T. Checkai, and R.S. Wentsel. 1993. Hexachloroethane obscurant: Assessing tree foliage injury. Environ. Tox. Chem., 12(4)685-694.

Skelly, J.M. 1990. Open-top chambers for ecological assessments. Evaluation of the effects of Army smokes/obscurants on forest tree species and "natural" vegetation. Final Report. Battelle Research, Research Triangle Park, NC.

Technique Name: **Chlorophyll Fluorescence Bioassay**

Technique Type: Photosynthesis Inhibition Bioassay
Matrix Type: Terrestrial and Wetland Plants
Ecosystem Level: Organismal
Test Location: Growth Chamber or Field

Description:

Impaired photosynthetic function caused by stresses due to soil contamination may be indicated by abnormal fluorescence patterns relative to plants inhabiting uncontaminated soils. A transportable fluorometer dedicated to analysis of photosynthesis is used in the field or at a fixed laboratory. Intact leaves or leaf segments are placed with the adaxial surface facing an actinic light source in the fluorometer. After dark adaptation (generally less than two minutes), fluorometric analyses are initiated. Fluorescence profiles may be plotted on an X-Y recorder, or electronic data may be stored in a data logger for later analysis. Variable and maximum fluorescence values [F_v and F_{max}] are measured from these fluorescence profiles, and plant health is in part described on the basis of derived ratio estimators based on F_v and F_{max} for short-term (0-30 sec) analyses. Longer-term tests (30 seconds to six minutes) have been performed to determine fluorescence decay over time. Fluorescence bands with maxima at 440 nm, 685 nm, and 740 nm have been measured.

Logistical Considerations:

Sample Collection:

Training: MINIMAL.

Time: MINIMAL.

Equipment: A portable fluorometer and oscilloscope, datalogger, or computer is required.

Sample Analysis:

Training: MINIMAL to extrapolate and calculate endpoints.

Time: MINIMAL.

Equipment: A computer is required to calculate endpoints.

Critique/Comments:

Chlorophyll fluorescence analysis is a fast, simple method for intact plants. If considered alone, photosynthesis or fluorescence may express sufficient variability (i.e., diurnal, seasonal, age-related, and non-contaminant related effects) to confound interpretations regarding adverse ecological effects. However, if completed in conjunction with whole-plant studies, altered fluorescence profiles in exposed plants may lend credence to interpretations of adverse ecological effects on the basis of site-specific empirical data. Also, compensatory mechanisms common to plants, e.g., reallocation of resources between plant organs in response to contaminant stress, may make extrapolation from leaf-level measurements to whole-plant and plant community-level interpretations difficult. Good control plants are essential.

The method(s) outlined and summarized here are early in the standardization process, and are not intended to be "stand alone" tests. Rather, the strengths of these method(s) lie in their contribution to weight of evidence arguments that are supportive of those methods that clearly illustrate adverse effects associated with soil exposures, e.g. early survival and vegetative vigor studies.

Key References:

Chappelle, E.W. and D.L. Williams. 1987. Laser induced fluorescence (LIF) from plant to foliage. IEEE Transactions on Geoscience and remote sensing. GE-25(6)726-736.

Judy, B.M., W.R. Lower, F.A. Ireland, and G.F. Krause. 1991. A seedling chlorophyll fluorescence toxicity assay. In Plants for toxicity Assessment: Second Volume, ASTM STP 1115. J.W. Gorsuch, W.R. Lower, W. Wang, and M.A. Lewis, eds. American Society for Testing and Materials, Philadelphia, PA. pp 146-158.

Lichtenthaler, H.K., ed. 1988. Applications of Chlorophyll Fluorescence in Photosynthesis Research, Stress Physiology, Hydrobiology, and Remote Sensing. Kleuver Academic Publishers, Dordrecht, The Netherlands.

Miles, D. 1990. The role of chlorophyll fluorescence as a bioassay for assessment of toxicity in plants. In W. Wang, J.W. Gorsuch, and W.R. Lower (eds). Plants for Toxicity Assessment. ASTM STP 1091. American Society for Testing and Materials, Philadelphia, PA. pp. 297-307.

NRC (National Research Council). 1989. Biologic markers of air-pollution stress and damage in forests. National Academy Press, Washington, D.C.

Technique Name: Aquatic Organism Toxicity Tests

Technique Type: Aquatic Bioassay
Matrix Type: Groundwater, surface water, soil or sediment eluate
Ecosystem Level: Individual
Test Location: Laboratory

Description:

Various aquatic organisms from many different trophic levels have been used to assess the effects of contaminants in aquatic ecosystems. Species of fish, macroinvertebrates, algae, and zooplankton are commonly used in bioassays. Organisms are added to a dilution series of site surface water, groundwater, or soil/sediment eluate. At the end of a given exposure period (usually 24, 48, or 96 h, depending on the species) for acute exposure or seven days for chronic exposure, relevant endpoints are measured and statistically calculated. Algal toxicity tests are conducted by adding cells of Selenastrum capricornutum to a series of concentrations of site surface water, groundwater, or soil/sediment eluate. Test chambers are incubated for 96 h under specific lighting conditions. At the end of the test period, cells are counted to determine measures of algal biomass and mean cell volume.

Logistical Considerations:

Sample Collection:

Training: MINIMAL

Time: MINIMAL

Equipment: MINIMAL for tests with surface water. A centrifuge is required for eluate extraction from sediment and soil.

Sample Analysis:

Training: MINIMAL

Time: MINIMAL

Equipment: A microscope, spectrophotometer, or electronic particle counter is needed to quantify algal cells.

Critique/Comments:

These tests have been extensively researched and validated for use in the field of environmental assessments. A large body of information is available documenting the ability of these tests to confirm the existence of adverse ecological effects. Unicellular algae function as primary producers and as such are important components of the aquatic ecosystem. Algal communities may be inhibited or stimulated by water quality changes. Cladoceran species -- e.g. Daphnia magna, Daphnia pulex, Ceriodaphnia dubia -- are the most common invertebrate species used. Fish tests typically involve species such as fathead minnows (Pimephales promelas), bluegills (Lepomis macrochirus), or rainbow trout (Oncorhynchus mykiss). These tests have been developed for use with a broad range of organisms beyond the few listed here. The American Society for Testing and Materials and the American Public Health Association have established testing guidelines for many species and for variations of the described methods.

Key References:

APHA, AWWA, WPCF. 1989. Part 8000 Toxicity test methods for aquatic organisms, In L.S. Clesceri, A. E. Greenberg and R.R. Trussel, eds., Standard Methods for the Examination of water and wastewater, 17th ed. American Public Health Association, Washington, D.C. pp. 8-1 through 8-143.

American Society for Testing and Materials (ASTM). 1994. Standard practice for conducting acute toxicity tests on aqueous effluents with fishes, macroinvertebrates, and amphibians, ASTM Committee E-47, American Society for Testing and Materials, Philadelphia, PA.

Blanck, H., and B. Bjornsater. 1989. The algal microtest battery: a manual for routine test of growth inhibition. KEMI Science and Technology Department Report, No. 3/89.

Environment Canada. 1992. Biological test method: growth inhibition test using the freshwater alga Selenastrum capricornutum. Conservation and Protection. Ottawa, Ontario, Canada. Environmental Protection Series, Draft Report (Jan.) 42p.

Horning, W.B., and C.I. Weber. 1985. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA/60/4-85/014. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency , Cincinnati, OH.

Linder, G., J. Wyant, R. Meganck, and B. Williams. 1991. Evaluating amphibian responses in wetlands impacted by mining activities in the western United States. In R.D. Comer, P.R. Davis, S.Q. Foster, C.V. Grant, S. Rush, O. Thorne, and J. Todd (eds.). Issues and technology in the management of

impacted wildlife. Thorne Ecological Institute. Boulder, CO. Pp. 17-25.
Peltier, W. and C.I. Weber. 1985. Methods for Measuring the Acute Toxicity of Effluents to Aquatic Organisms. Third Edition. EPA/600/4-85/013. Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH.

Technique Name: Sediment Toxicity Tests

Technique Type: Aquatic Bioassay
Matrix Type: Freshwater Sediment
Ecosystem Level: Individual
Test Location: Laboratory

Description:

Test organisms are added to chambers containing contaminated sediment and control water in a 1:4, v/v, ratio of sediment and water. At the end of the test, one of a wide variety of endpoints is measured. Test organisms commonly used in sediment toxicity tests include bacteria, rotifers, nematodes, periphyton, pelecypods, oligochaetes, cladocerans, isopods, amphipods, insects, fish, amphibians, and macrophytes. Sediment toxicity assessments can be conducted using acute or chronic exposures using one of a variety of endpoints depending on exposure period and test organism. Typical endpoints include survival, growth, molting frequency, reproduction, enzyme activity, avoidance, embryo-larval survival, adult emergence, and luminescence.

Logistical Considerations:

Sample Collection:

Training: MINIMAL

Time: MINIMAL

Equipment: MINIMAL

Sample Analysis:

Training: MODERATE

Time: MINIMAL

Equipment: MINIMAL

Critique/Comments:

A serious need exists for the development of standard sediment toxicity assessment procedures. The procedure described above varies considerably from some other accepted methods currently used. Test species should be selected based on their behavior in the sediment, their sensitivity to chemical and physical

parameters of the sediment, their availability, their sensitivity to the contaminant, and sediment phase tested. A large amount of literature deals with the subject of species selection. Benthic organisms serve as excellent overall indicators of aquatic contaminant effects for several reasons. Benthic organisms are integrally associated with the sediment and interstitial waters. The sensitivity of many species to common pollutants is well documented. Several well developed assays have proven effective in detecting sediment toxicity.

One of the most important aspects of sediment toxicity assessment is selection of the proper sediment phase to test. Phases of sediment are extractable phase, elutriate phase, interstitial water phase, and whole sediment. The elutriate phase contains contaminants extractable by water. Since many contaminants are not removed by water, other solutes may be employed to extract contaminants in the extractable phase. The interstitial water phase is the interstitial water from the sediment. The whole sediment is the sediment sample collected from the contaminated waste site with as little manipulation as possible.

Key References:

American Society for Testing and Materials. 1994. ASTM standards on Aquatic Toxicology and Hazard Evaluation. American Society for Testing and Materials, Philadelphia, PA.

Burton, G.A., Jr. 1991. Assessing the toxicity of freshwater sediments. Environ. Toxicol. Chem. 10:1585-1627.

Burton, G.A., Jr., M.K. Nelson and C.G. Ingersoll. 1992. Freshwater benthic toxicity tests, In G.A. Burton, ed., Sediment Toxicity Assessment. Lewis Publishers, Boca Raton, FL, pp 213-240.

Giesy, J.P. and R.A. Hoke. 1989. Freshwater sediment toxicity bioassessment: rationale for species selection and test design. J. Great Lakes Res. 15:539.

Hill, I.R., Matthiessen, P, and F. Heimbach, eds. 1994. Guidance document on sediment toxicity tests and bioassays for freshwater and marine environments. Society of Environmental Toxicology and Chemistry-Europe. 105 pp.

Technique Name: **Amphibian Test Methods**

Technique Type: Survival, Growth, Teratogenesis
Matrix Type: Amphibian (embryos)
Ecosystem Level: Organismal
Test Location: Laboratory

Description:

Amphibian testing uses FETAX (frog embryo teratogenesis assay: Xenopus laevis). Tests may be completed with surface waters, groundwater, or soil/sediment-derived eluates. To initiate exposures, less than eight-hour old frog embryos are placed in Petri dishes containing aqueous test solutions for either screening or definitive tests. In definitive tests triplicate exposure series are set up with a maximum of five to six concentrations plus controls in each replicate. For screening purposes, triplicate Petri dishes may contain 100% site-samples. Once exposures have been initiated, the 96-hour static-replacement exposures are renewed at 24-hour intervals at 22±2°C. Endpoints include survivorship, growth (e.g., length), and malformations observations. Survivorship data (LC_{50} or percent survival in 100% site-sample) is determined at the end of four-day exposures. Similarly, EC_{50}s for malformation are recorded in definitive tests, or percent malformations is recorded in screening tests. Subacute response data will reflect numbers of gross terata (e.g., scoliosis, lordosis, and kyphosis) developed in exposed embryos.

Logistical Considerations:

Sample Collection:

Training: MINIMAL to expose and maintain organisms.

Time: MINIMAL (96-hr).

Equipment: MINIMAL to maintain embryos.

Sample Analysis:

Training: MINIMAL.

Time: MINIMAL.

Equipment: MINIMAL.

Critique/Comments:

Amphibian test systems are standardized through ASTM (American Society for Testing and Materials). Early embryos of the African clawed-frog (Xenopus laevis) are used in the standardized test; however, much work has been completed with alternative test species and should be considered on a site-specific basis. The test method was originally designed for testing surface waters and water column exposures with sediments. At present the method is most directly applicable to wetland evaluations that may be required as part of an ecological effects assessment.

Care must be taken to correctly determine the degree of ecological significance that may be derived from these tests. Unless in situ methods are also included as part of the ecological effects assessment "laboratory to field" extrapolation error may confound biological assessments within an ecological risk context. While more laboratories are offering testing services with amphibians, only a limited number of technical support laboratories are currently providing tests with these organisms.

Key References:

ASTM E729. 1991. Standard guide for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. Annual Book of Standards, American Society for Testing and Materials, Philadelphia, PA.

ASTM E1439. 1991. Standard guide for conducting the frog embryo teratogenicity test: Xenopus. Annual Book of Standards, American Society for Testing and Materials, Philadelphia, PA.

Adamus, P.R. and K. Brandt. 1990. Impacts on quality of inland wetlands of the United States: A survey of indicators, techniques, and applications of community level biomonitoring data. (EPA/600/3-90/073). U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, Oregon, 97333.

Linder, G., J. Wyant, R. Meganck, and B. Williams. 1991. Evaluating amphibian responses in wetlands impacted by mining activities in the western United States. In R.D. Comer, P.R. Davis, S.Q. Foster, C.V. Grant, S. Rush, O. Thorne, and J. Todd (eds.). Issues and technology in the management of impacted wildlife. Thorne Ecological Institute. Boulder, CO. Pp. 17-25.

Technique Name: Static Microalgae Toxicity Test

Technique Type: Growth Assay
Matrix Type: Green Algae
Ecosystem Level: Organismal
Test Location: Laboratory

Description:

Algae are cultured in nutrient media. Culture in log phase growth ($\geq$ 2 X 10^4 cells/mL) are placed into erhlenmyer flasks containing reagent grade laboratory chemicals. The preferred solvent is dilution water. However, if an organic solvent is necessary, triethylene glycol is recommended because of its low volatility and high ability to dissolve organic chemicals. Methanol, ethanol, and acetone may also be used, but they might stimulate undesirable growth of algae and microorganisms. The concentration of an organic solvent should be $\leq$ 0.5 mL/L. Treatments consist of one or more controls and a geometric series of at least five concentrations of test material. Temperature and illumination should be controlled and will differ depending on the test organism. Several organisms are recommended depending upon the type of habitat being studied. For freshwater studies, the green algae Selanastrum capricornutum is most widely used, however other green and blue green algae, and diatoms have been used successfully. The diatom Skeletonema costatum is most commonly used for saltwater samples. Test endpoints are biomass and 96-hour IC_{50} based on reduction of growth.

A modified version of this test uses microplates (220 ul, 10,000 cells/mL) rather than flasks, incorporates 9 concentrations, and has a duration of 72 hrs (Environment Canada, 1992. Blanck and Bjornsater, 1989). This test may be more amenable to testing effluent receiving water, leachates, and elutriates. Endpoints include LOEC and NOEC as well as IC_{50}.

Logistical Considerations:

Sample Collection:

Training: MINIMAL.

Time: MINIMAL.

Equipment: Controlled temperature and lighting are required.

Sample Analysis:

Training: MINIMAL.

Time: MINIMAL.

Equipment: MINIMAL.

Critique/Comments:

These tests provide information on the toxicity of test materials to an important component of the aquatic biota and might indicate whether longer term tests are desirable. The tests may also be used to study biological availability of, and structure-activity relationships between, test materials. These procedures are applicable to many chemicals, either individually or in formulations, commercial products, or known mixtures. With appropriate modifications, these tests can be used to measure the effects of temperature, dissolved oxygen, and pH on such materials as aqueous effluents, leachates, soils, particulate matter, sediments, and surface waters. Static tests might not be applicable to materials that have a high oxygen demand, are highly volatile, are rapidly transformed in aqueous solutions either biologically or chemically, or are removed from test solutions in substantial quantities by the test chambers or organisms during the test.

Key References:

ASTM. 1992. Standard guide for conducting static 96-hour toxicity tests with microalgae, designation E1218-90. p 874-885. In Annual Book of ASTM Standards, Section 11, Water and Environmental Technology, Volume 11.04.

Blanck, H., and B. Bjornsater. 1989. The algal microtest battery: a manual for routine test of growth inhibition. KEMI Science and Technology Department Report, No. 3/89.

Environment Canada. 1992. Biological test method: growth inhibition test using the freshwater alga Selenastrum capricornutum. Conservation and Protection. Ottawa, Ontario, Canada. Environmental Protection Series, Draft Report (Jan.) 42p.

Miller, W.E., J.C. Greene, and T. Shiroyama. 1978. Selenastrum capricornutum Printz Algal Assay Bottle Test: Experimental Design, Application, and Data Interpretation Protolcol. EPA-600/9-78-018, Corvallis, OR.

U.S. Environmental Protection Agency. 1974. Marine Algal Assay Procedure: Bottle Test. National Environmental Research Center, Corvalis, OR.

Weber, C.I., ed. 1973. Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents. Environmental Monitoring and Support.

Technique Name: **Early Life Stage Fish Toxicity Test**

Technique Type: Survival, Growth Assay
Matrix Type: Freshwater and Saltwater Fish
Ecosystem Level: Organismal
Test Location: Laboratory

Description:

The fish in this test are newly fertilized (uneyed) embryos (≤ 24 h after fertilization). Recommended species for such tests include: salmon, trout, char, Northern pike, fathead minnow, white sucker, channel catfish, bluegill, sheepshead minnow, and silversides. Fish are cultured in flow-through tanks and incubation cups may be used. Temperature and aeration should be monitored and controlled, with air filtration using a 0.22 um bacterial filter recommended. To reduce stress, organisms should be shielded with partitions or curtains. Use of a "non-toxicant" test in which organisms are placed in dilution water to determine survival and growth survivability is recommended. The dilution water source is recommended to be reconstituted water or uncontaminated natural dilution water for early-stage toxicity tests. The test material should be dissolved in the dilution water before adding.

The tests usually consist of at least one control treatment and a geometric series of at least five concentrations of test material. The length of the tests will vary greatly dependent primarily upon the time required for hatching which varies with the species. Tests are generally terminated within 28 days after exposure to the test material, depending upon the purpose of the test. The most common endpoints of these tests are determination of mortality, number, and growth. The mortality is commonly measured at selected times using LC_{50} values, while the growth endpoint uses NOEC, LOEC, IC_p values. Endpoint values such as EC10, EC25, and EC50 may be used for both.

Logistical Considerations:

Sample Collection:

Training: MODERATE, experience in obtaining egg and sperm from adult fish may be required for some fish species as well as handling and monitoring of health may be needed. Such training typically would require several weeks.

Time: MODERATE, up to 47 days for some species.

Equipment: MODERATE cost to setup, possibly in the $10,000 rand including monitoring equipment. MINIMAL if a lab already possesses equipment.

Sample Analysis:

Training: MINIMAL to record survivability and weight.

Time: MINIMAL to MODERATE depending upon the species of fish.

Equipment: MINIMAL for most visual endpoints, an analytical balance is needed to record weights.

Critique/Comments:

These tests are generally used to provide data on the toxicity of test materials of varying concentrations compared to controls. They can be used to determine embryo survival, fry survival, overall survival, and weight of the survivors in each treatment. These tests are applicable to all chemicals, either individually or in formulations, commercial products or known mixtures, that can be measured accurately at the necessary concentrations of water. With appropriate modifications, these procedures can be used to conduct tests on the effects of temperature, dissolved oxygen, and pH, and such materials as aqueous affluents, leachates, oils, particulate matter, sediments, and surface waters. Results of these tests may also be used to predict long-term effects likely to occur on fish in field situations, except that mobile organisms might avoid exposure when possible. Another possible use of these tests is to assess hazards to aquatic organisms when deriving water quality criteria.

Some species of fish, particularly striped bass, silversides, and trout, are difficult to handle without proper training and may have high mortality rates after hatching. The validity of test results may be placed in question if the rate of survivability after hatching exceeds 70%. Thus, much time in setup and determining ideal living conditions for the fish may be needed.

Key References:

Keddy, C., J.C. Greene, and M.A. Bournell. The National Contaminated Sites Remediation Program, October 1992. Prepared for CCME Subcommittee on Environmental Quality Criteria for Contaminated Sites.

Annual Book of ASTM Standards, Section 11, Water and Environmental Technology, Vol. 11.04. Designation E1241-92.

Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fishes, pp. 886-913.

Technique Name: Conducting Three Brood, Renewal Toxicity Tests with Ceriodaphnia dubia

Technique Type: Survival, Growth, Reproductivity, and Physiological Response
Matrix Type: Invertebrate, Crustacea, Ceriodaphnia dubia
Ecosystem Level: Organismal
Test Location: Laboratory

Description:

Survival, growth, reproductivity, and physiological response endpoints can be obtained from Ceriodaphnia dubia exposed to the effects of an effluent or test material added to test material, but not food, during a portion of the organisms life. C. dubia are easily cultured in small (30-50 ml) covered test chambers of glass or plastic construction. Natural freshwater from an uncontaminated source or reconstituted water may serve as the dilution water. Aeration (between 40 and 100% saturation) and temperature must be controlled and monitored. Organisms should be less than 12 h old and are easily obtainable from commercial supply houses. A three brood toxicity test intended to allow calculation of an endpoint (usually a reduction in number of live neonates produced by first-generation C. dubia) usually consists of one or more control treatments and a geometric series of at least five concentrations of test material or effluent. Point estimates such as EC10, EC25, and EC50 may be used when using regression analysis. LC_{50} may also be an applicable endpoint for survivability.

Logistical Considerations:

Sample Collection:

Training: MINIMAL for culturing and feeding.

Time: MINIMAL (<3 days).

Equipment: MINIMAL for culture of C. dubia.

Sample Analysis:

Training: MINIMAL to determine endpoints.

Time: MINIMAL to determine endpoints.

Equipment: MINIMAL, sensitive balance for determining weight, dissection

microscope for detecting physiological response and measuring length.

Critique/Comments:

Daphnids such as C. dubia are easily obtained and cultured. Their sensitivity to a variety of test materials makes them ideal organisms for freshwater aquatic tests. Applicability to field conditions indicate a high correlation with laboratory test. C. dubia is preferable to Daphnia magna because of its shorter generation time (tests can be carried out in only 4-7 days as opposed to 21 days required for D. magna). The 7-day test is preferred by Oris et al (1991) because of its sensitivity to both individual substances and complex effluents. One drawback to using C. dubia, is that behavioral and developmental effects are difficult to quantify and may not provide meetable endpoints.

Key References:

ASTM E1295. 1989. Standard Guide for Conducting Three-Brood, Renewal Toxicity Tests with Ceriodaphnia dubia. Annual Book of ASTM Standards. Vol. 11.04. Philadelphia, PA.

Mount, D.I., and T.J. Norberg. 1984. "A Seven-Day Life-Cycle Cladoceran Toxicity Test," Environmental Toxicology and Chemistry, Vol. 3, pp. 425-434.

Oris, J.T., A.T. Hall, and J.D. Tylka. 1990. "Humic Acids Reduce the Photo-Induced Toxicity of Anthracene to Fish and Daphnia." Environmental Toxicology and Chemistry, Vol. 9, pp. 575-583.

Technique Name: **Earthworm Survival and Sublethal Effects**

Technique Type: Survival, Growth, Physiological Response
Matrix Type: Invertebrate, Earthworms
Ecosystem Level: Organismal
Test Location: Laboratory, Field

Description:

Methods directly evaluate the biological effects of contaminated soils on a representative macroinvertebrate (Eisenia foetida, E. andrei, or Lumbricus terrestris). In 14 day screening tests, percent survival is recorded at day 7 and day 14. Mortality is the most frequently measured end point in definitive tests; although growth, behavioral, and pathogenic observations may also be recorded. Median lethal concentrations (LC_{50}'s) and 95% confidence intervals are calculated at day 7 and day 14. Positive control LC_{50}'s (with 2-chloroacetimide) should be completed for definitive survival tests. Sublethal endpoints include one or more of the following: weight loss, dermopathologic responses, muscular responsiveness, presence or rate of burrowing, and reproduction. Analysis of covariance (ANCOVA) is recommended for weight loss studies. A similar test with an alternative soil annelid (Enchytraeus albidis) should be considered when the physicochemical properties of a test soil (e.g. moisture fraction, temperature) are not conducive to a successful test with E. foetida. Four-week tests with E. albidis measure mortality and biomass end points; eight-week tests measure offspring production.

Logistical Considerations:

Sample Collection:

Training: MINIMAL to raise earthworms, treat soils, and record growth and survival.

Time: MODERATE for earthworms (14-30 days) and enchytraeids (30-60 days).

Equipment: Cost is MINIMAL. Tests can be purchased or materials bought separately. Coolers, media and feed are required to raise earthworms. Tests can be performed in jars or beakers in temperature and light controlled chamber or room.

Sample Analysis:

Training: Training is MINIMAL to record survivability, weight, and cocoon production, MINIMAL to MODERATE for recognizing physiological responses.

Time: Time is MINIMAL to measure necessary endpoints.

Equipment: Equipment is MINIMAL for most visual observations. An analytical balance is required for weight endpoints.

Critique/Comments:

Earthworm tests are commercially available, cost-effective, simple, relatively short-term, and reliable. Methods for survival and/or growth have been published by International Standards Organization (ISO), OECD and EPA. Requirements for routinely using earthworm test methods are outlined in USEPA (1989), as well as the applied ecology literature (e.g. Callahan et al, 1985 and Neuhauser, et al 1986). These tests may be useful in integrated studies that include laboratory and in situ toxicity evaluation. Assessments with earthworms can help address site-specific issues related to bioavailability of contaminants. Survival and growth endpoints are relatively easy to implement, but behavioral and pathological endpoints require special training. Soil characteristics (e.g. strongly acidic, strongly alkaline, wetlands, nutrient deficient) at some sites may be incompatible with earthworms. The selection of test species must be given ample consideration during the problem formulation phases of the ecological assessment. Adequate characterization of the soil matrix prior to toxicity testing can minimize "false negatives" that may result from selection of an inappropriate test species.

Key References:

Callahan, C.A., L.K. Russell, and S.A. Peterson. 1985. A comparison of three earthworm bioassay procedures for the assessment of environmental samples containing hazardous wastes. Biol. Fert. Soils. 1:195-200.

Callahan, C.A., C.A. Menzie, D.E. Burmaster, D.C. Wilborn, and T. Ernst. 1991. On-site methods for assessing chemical impact on the soil environment using earthworms: a case study at the Baird and McGuire Superfund site, Holbrook, Massachusetts. Environ. Toxicol. Chem. 10:817-826.

Lofs-Holmin, A. 1980. Measuring growth of earthworms as a method of testing sublethal toxicity of pesticides. Swedish J. Agric. Res. 10:25-33.

Neuhauser, E.F., P.R. Durkin, M.R. Milligan, and M. Anatra. 1986. Comparative toxicity of ten organic chemicals to four

earthworm species. Comp. Biochem. Physiol. 83C(1):197-200.
Rombke, J. 1989. Enchytraeus albidus (Enchytreidae, Oligochaeta) as a test organisms in terrestrial laboratory systems. Arch. Toxicol., Suppl. 13:402-405.
US EPA. 1989. Protocols for short term toxicity screening of hazardous waste sites. J.C. Greene, C.L. Bartels, W.J. Warren-Hicks, B.R. Parkhurst, G.L. Linder, S.A. Peterson, and W.E. Miller (eds.). EPA/600/3-88/029, U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, OR.
Wentsel, R.S. and M.A. Guelta. 1987. Toxicity of brass powder in soil to the earthworm Lumbricus terrestris. Environ. Toxicol. Chem. 6:741-745.

Technique Name: **Free-Living Nematode Survival and Sublethal Effects**

Technique Type: Survival, Growth, Physiological Response
Matrix Type: Invertebrate, Nematodes
Ecosystem Level: Organismal
Test Location: Laboratory

Description:

Survival, growth, reproduction and mutagenicity of Panagrellus redivivus or Caenorhabditis elegans exposed to contaminated soils can be measured using a short-term (4-7 day) test. P. redivivus, an aquatic species with a well-developed database in aquatic toxicity testing, has been applied to sediment, and can be applied to soil toxicity testing. A more recent test using C. elegans, a soil-dwelling nematode, may be applicable for ecological effects assessments. Tests with this nematode may more accurately reflect soil contaminant effects in terrestrial habitats. Comparative analysis of LC_{50}'s of C. elegans, Daphnia magna, and sediment macroinvertebrates exposed to aqueous solutions from soil sediments contaminated with metals showed acute toxicities among the three tests. Methods using free-living nematodes were designed as complimentary to earthworm tests.

Logistical Considerations:

Sample Collection:

Training: MINIMAL for culturing and treating nematodes.

Time: MINIMAL ($\leq$ 7 days).

Equipment: MINIMAL for culture of nematodes.

Sample Analysis:

Training: MINIMAL to determine endpoints.

Time: MINIMAL to determine endpoints.

Equipment: MINIMAL TO MODERATE. A compound light microscope is required.

Critique/Comments:

Nematodes afford many advantages for toxicity testing from a laboratory perspective. Nematodes frozen in liquid nitrogen can be preserved (-80°C) and rehydrated prior to testing. Standardized sampling strategies have been established and published by ASTM. Although developed for efficacy evaluations for nematode control agents, methods could easily serve the needs of ecological effects assessment. For soil contamination evaluations, relatively little work has been completed to address questions related to "laboratory to field" extrapolation errors, and few applied studies have been published regarding the effects of contaminant mixtures on soil community structure. P. redivivus is an aquatic species and would be applicable to indirect (i.e. assessing soil eluates) tests. C. elegans although a free living soil nematode, may not inhabit all soil types.

Key References:

ASTM E629. 1991. Standard guide for field evaluation of nematode control agents - determination of nematode population responses to control agents. Annual book of ASTM standards. Volume 11.04. Pesticides; Resource Recovery; Hazardous Substances and Oil Spill Responses; Waste Disposal; Biological Effects. American Society for Testing and Materials (ASTM). Philadelphia, PA 19103.

Samoiloff, M., S. Schulz, Y. Jordan, K. Denich, and E. Arnott. 1980. A rapid simple long-term toxicity assay for aquatic contaminants using the nematode Panagrellus redivivus. Can. J. Fish. Aquat. Sci. 37:1167-1174.

Samoiloff, M., J. Bell, D. Birkholz, G. Webster, E. Arnott, R. Pulak, and A. Madrid. 1983. Combined bioassay-chemical fractionation scheme for the determination and ranking of toxic chemicals in sediment. Environ. Sci. Technol. 17:329-333.

van Kessel, W., R. Brocades Zaalberg, and W. Seinen. 1989. Testing environmental pollutants on soil organisms: a simple assay to investigate the toxicity of environmental pollutants on soil organisms, using $CdCl_2$ and ematodes. Ecotoxicol. Environ. Safe. 18:181-190.

Technique Name: **Terrestrial Arthropods (insects)**

Technique Type: Survival, Growth, Reproduction
Matrix Type: Invertebrate, Insect
Ecosystem Level: Organismal
Test Location: Laboratory or Field

Description:

Various methods have been developed for evaluating chemical effects on terrestrial insects, especially pesticide effects on nontarget species. Survival is the most commonly measured endpoint, although other acute and chronic endpoints have been used. One standardized method for evaluating acute and subacute chemical effects on terrestrial insects was initially developed for agrichemical evaluations, especially for evaluating the effects of insecticides on non-target insects (e.g., honey bees (apis mellifera); see US EPA 1982). Crickets and harvester ants have been used on a limited basis within an ecological effects assessment. Adult house crickets (Acheta domesticus) were exposed to acridine via the diet, and following 18-day exposures lethality and sublethal effects were determined. House crickets and field crickets (Gryllus pennsylvanicus), have been used to show lethal effects (LC_{50}) and bioaccumulation of PCB's in a field situation (Burrow, et. al., 1993). Harvester ants (Pogonomyrmex owyheei) have proven sensitive to some organic contaminants (i.e., pesticides) and complex chemical mixtures (e.g., wood preservative sludge, drilling fluid, and slop oil). Genotoxicity screening with Drosophila melanogaster (fruit fly) may be applicable if it is considered to be a representative hymenopteran.

Logistical Considerations:

Sample Collection:

Training: MINIMAL.

Time: MODERATE (7-21 days).

Equipment: MINIMAL to culture insects and treat soils for laboratory tests and MINIMAL to construct traps for field tests.

Sample Analysis:

Training: MINIMAL to record endpoints.

Time: MINIMAL.

Equipment: MINIMAL TO MODERATE. A dissecting light microscope is required for some assays.

Critique/Comments:

As ecological indicators of soil contamination, terrestrial insects, and soil arthropods in general, are potentially critical organisms. These test guidelines were all designed with agrichemicals or other challenging agents (chemical or biological) as potential hazards. However, in evaluating adverse biological effects, test design may be similar regardless of the agent. And, while toxicity estimates may be derived from modifications of these existing tests, the interpretation of the toxicity information should be weighted by site-specific information gathered, for example, during field surveys. It is critical that issues regarding the interpretation of toxicity test data be addressed early in the problem formulation phase of the ecological effects assessment.

Few technical support laboratories are currently providing tests with insects, particularly within the context of hazardous waste sites, but technical support may be gained on a site-specific basis, e.g., through local or regional testing services available at land-grant colleges. Until adequate technical support is available, implementing this biological assessment within an ecological effects assessment may be difficult. Additionally, the data base is relatively sparse; use of adequate reference soils for site-specific comparisons is critical.

Key References:

Croft, B.A. 1990. Arthropod biological control agents and pesticides. John Wiley & Sons, New York, NY. 723 pp.

Gano, K.A., D.W. Carlile, and L.E. Rogers. 1985. A harvester ant bioassay for assessing hazardous chemical waste sites. PNL-5434, UC-11. Pacific Northwest Laboratory, Richland, WA.

OECD (Organization for Economic Co-Operation and Development). 1984. OECD guidelines for testing of chemicals. Director of Information, OECD. 2, rue Andre Pascal, 75775 Paris Cedex 16, France.

US EPA. 1982. Pesticide assessment guidelines, Subdivision L, Hazard Evaluation: Non-target insects. 540/9-82/019. Office of Pesticides and Toxic Substances, U.S. Environmental Protection Agency, Washington, D.C.

Walton, B.T. 1980. Differential life-stage susceptibility of Acheta domesticus to acridine. Environ. Entomol. 9:18-20.

Technique Name: **Terrestrial Arthropods (non-insect) and isopods**

Technique Type: Survival, Reproduction, Genotoxicity
Matrix Type: Invertebrate, Arthropod (non-insect), and Isopod
Ecosystem Level: Organismal
Test Location: Laboratory

Description:

Test systems are well characterized although exposures are generally not associated with soil exposure directly. Within an ecological effects assessment, however, test systems may be easily modified to assure that exposures occur directly via site-soil (for example, contained in Petri dishes). Alternatively, exposures could occur via glass plates or Petri dishes coated with dried films of single-compound, defined chemical mixture, or soil eluate. Acute toxicity has been the most easily measured endpoint following exposure periods that range from nearly one week to four weeks. Additional endpoints should also consider reproductive success in the test species; most frequently achieved by counting the number of eggs laid during the exposure period. A springtail (Folsomia Candida) test has been adopted as a draft test method by the International Standards Organization (ISO). Endpoints for this 4-week test include adult survival, offspring number, NOEC and LOEC. Testing of pure chemical or soil eluates occurs in whole artificial soil under controlled temperature, light intensity, and photoperiod.

Although the distribution of microarthropods has not been routinely used to assess soil toxicity, this approach could be used in hazardous waste site assessments. The microarthropods are extracted from the contaminated and uncontaminated soils using Berlese funnels or high intensity Tullgren extractors. The extracted arthropods should be preserved for effects-based comparisons (e.g., total numbers and identification).

Logistical Considerations:

Sample Collection:

Training: MINIMAL to maintain organisms in soil.

Time: MINIMAL to MODERATE (7-day to 4-wk) extractors can be built with MINIMAL expense.

Equipment: MODERATE. An environmentally-controlled room or chamber is required to regulate

temperature and light throughout the test.

Sample Analysis:

Training: MINIMAL to MODERATE for inexperienced personnel. However, training should be performed by someone with EXTENSIVE training.

Time: Sampling time is MINIMAL to MODERATE depending on experience of sampler and the number of samples.

Equipment: Sampling equipment is MODERATE. A dissecting light microscope is required.

Critique/Comments:

Because of their role in the environment, terrestrial arthropods and isopods should receive consideration as an ecological "receptor" during the ecological assessment within the RI/FS process. By comparison of populations in contaminated and uncontaminated site soils, it will be possible to demonstrate population shifts corresponding to soil toxicity. In addition, information on specific community responses to specific chemicals and chemical mixtures must be developed.

Determination of the impacts of specific chemicals on specific populations of microarthropod or isopods have generally been limited, although the applied literature indicates that species-specific sensitivities may be expressed, e.g., copper effects on isopods. Expertise in the identification of microarthropods, and determination of their numbers exists within most land-grant universities, the USDA, and the extension service, either at the Federal or State level. Regional centers of expertise have been suggested although few technical support laboratories currently provide these tests. From a technical perspective, terrestrial arthropods in general and non-insects in particular have a poorly established comparative effects and toxicity database. While the potential strengths associated with toxicity evaluations and effects measurements are numerous (e.g., more ecologically relevant, amenable to laboratory and field assessment), the lack of commercial availability may also limit the routine application of these methods in an ecological effects assessment.

Key References:

Anderson, J.M. 1988. Spatiotemporal effects of invertebrates on soil processes. Biol. Fertil. Soils. 6:216-227.

Hassan, S.A. 1985. Standard methods to test the side-effects of pesticides on natural enemies of insects and mites developed

by the IOBC/WPRS work group 'Pesticides and beneficial organisms.' Bull. OEPP/EPPO 15:214-255.
Hassan, S.A., R. Albert, F. Bigler, P. Blaisinger, H. Bogenschutz, E. Boller, J. Brun, P. Chiverton, P. Edwards, W.D. Engloert, P. Huang, C. Inglesfield, E. Nation, P.A. Oomen, W.P.J. Overmeer, W. Rieckmann, L. Samsoe-Petersen, A. Staubli, J.J. Tuset, G. Viggiani, and G. Vanwetswinkel. 1987. Results of the third joint insecticide testing programme by the IOBC/WPRS-working group "Pesticides and beneficial organisms." J. Appl. Ent. 103:92-107.
Hopkin, S.P. 1986. The woodlouse Porcellio scaber as a 'biological indicator' of zinc, cadmium, lead and copper pollution. Environ. Pollut. (Series B) 11:271-290.
Moldenke, A.R. and B.L. Fichter. 1988. Invertebrates of the H.J. Andrews Experimental Forest, Western Cascade mountains, Oregon: IV. The oribatid mites (Acari; Cryptostigmata). USDA Forest Service. PNW-GTR-217.

Technique Name: **Invertebrate Immunotoxicity**

Technique Type: Immunotoxicity
Matrix Type: Invertebrate, Earthworm
Ecosystem Level: Individual
Test Location: Laboratory

Description:

Although immunotoxicity test methods are more widely described for vertebrates, similar methods have been applied to terrestrial invertebrates under controlled laboratory conditions. For example, methods have been developed for evaluating the immunocompetence of earthworms (generally Lumbricus terrestris). In these subacute tests, earthworms are exposed to single-chemicals or complex mixtures. The majority of work has been completed using filter-paper contact tests, but soil exposed-earthworms could be tested in conjunction with laboratory or in situ tests. A biomarker, altered immune function in earthworms, should be considered as supporting data in an integrated study that addressed, for example, organismic-level measurements (e.g., standardized 14-day earthworm test; and field survey information. Although not sufficiently developed at present, the future value of these methods centers upon the comparative toxicity data base that can be developed relative to other receptors (e.g., mammals and birds).

Logistical Considerations:

Sample Collection:

Training: MODERATE to EXTENSIVE.

Time: MODERATE (7-14 days).

Equipment: MODERATE to collect leucocytes.
EXTENSIVE to collect and purify enzymes.

Sample Analysis:

Training: MODERATE to EXTENSIVE.

Time: MODERATE to conduct enzyme assays and immunoassays.

Equipment: MODERATE to EXTENSIVE.

Critique/Comments:

At present, the interpretation of altered immune function in soil

macroinvertebrates should be guarded, particularly within the context of an ecological effects assessment. Only through integrated studies using organismic-level tests and field surveys will results from these invertebrate immune function tests be ecologically relevant. However, unlike the immunotoxicity information garnered from terrestrial vertebrates, an evaluation of the immunocompetence of soil macroinvertebrates, such as earthworms, may directly reflect the long-term adverse biological effects associated with soil contaminants. For terrestrial vertebrates, the majority of exposure routes are indirect, except for direct soil ingestion, but for soil-dwelling invertebrates the routes of exposure are more direct due to the close contact between receptor and contaminant source. Cutaneous or dermal uptake of contaminants in earthworms are equal, if not greater, than direct soil ingestion depending upon physicochemical properties of the contaminant mixture at a site.

Measuring immunocompetence requires specialized training and the techniques can be time-consuming. Few technical support laboratories are currently providing tests with these organisms, and implementing these methods within an ecological effects assessment may be difficult due to an absence of experienced testing services.

Key References:

Chen, S.C., L.C. Fitzpatrick, A.J. Goven, B.J. Venables, and E.L. Cooper. 1991. Nitroblue tetrazolium dye reduction by earthworm (Lumbricus terrestris) coelomocytes: an enzyme assay for nonspecific immunotoxicity of xenobiotics. Environ. Toxicol. Chem. 10:1037-1043.

Enyambe, G.S., A.J. Goven, L.C. Fitzpatrick, B.J. Venables, and E.L. Cooper. 1990. A non-invasive technique for sequential collection of earthworm (Lumbricus terrestris) leukocytes during subchronic immunotoxicity studies. Lab. Animals 25:61-67.

Mohrig, W., E. Kanschke, and M. Ehleers. 1984. Rosette formation by coelomocytes of earthworm Lumbricus terrestris L. with sheep erythrocytes. Devel. Comp. Immunology 8:471-476.

Rodriguez-Grau, J., B.J. Venables, L.C. Fitzpatrick, and E.L. Cooper. 1989. Suppression of secretory rosette formation by PCBs in Lumbricus terrestris: an earthworm assay for humoral immunotoxicity of xenobiotics. Environ. Toxicol. Chem. 8:1201-1207.

Stein, E.A. and E.L. Cooper. 1988. In vitro agglutinin production by earthworm leukocytes. Devel. Comp. Immunol. 12:531-547.

Technique Name: Mollusks (Terrestrial and Wetland)

Technique Type: Survival, Transformation
Matrix Type: Invertebrates, Mollusks
Ecosystem Level: Organismal
Test Location: Laboratory

Description:

Anodonta imbecilis was initially selected as a representative unionid mollusk; however, the techniques should be applicable for testing mussels with similar reproductive strategies. Exposures are static or renewal, and depending upon endpoint (e.g., survival or transformation), the exposures are 24-hr or 9 to 11 days. All tests involve the early developmental stages of the mussel, or glochidia, and juvenile mussels, depending upon endpoints being measured. The method is applicable to assessments of wetland or aquatic habitats.

A similar method, originally developed for efficacy tests can be modified to assess terrestrial habitats. Laboratory reared snails (e.g., Derocera reticulatum) or slugs are exposed to contaminated soils in test boxes or glass aquaria for 24 to 48-hr. Endpoint is mortality, but the test duration could be lengthened to measure sublethal endpoints.

Logistical Considerations:

Sample Collection:

Training: MINIMAL to prepare media.

Time: MINIMAL to MODERATE (24-hr to 11-day).

Equipment: MINIMAL.

Sample Analysis:

Training: MINIMAL to measure growth and survival.

Time: MINIMAL.

Equipment: MINIMAL.

Critique/Comments:

Aquatic toxicity tests with freshwater mussels may be critical to a wetland evaluation if complex chemical mixtures characteristic of hazardous waste sites were impacting the habitat. Guidance for developing the test with freshwater mussels followed ASTM E729 (1991), and while not widely used at this time, toxicity assessments with freshwater mussels should be considered within an ecological effects assessment. Similarly, the test with terrestrial snails and slugs could complement a field survey. Although originally designed as an efficacy test, the method can be applied to evaluations of the effects associated with soil exposures.

Additionally, when threatened or endangered fresh water mussels are potential receptors at a Superfund site, these test methods should seriously be considered. No comparative toxicity data base has been developed, although the mollusk literature is widespread with an increasing amount of work being reported that summarize ecological effects associated with exposures involving freshwater and terrestrial mollusks. Only a few technical support laboratories are currently providing tests with these organisms.

Key References:

ASTM E729. 1991. Standard guide for conducting acute toxicity test with fishes, macroinvertebrates, and amphibians. Annual Book of Standards, American Society for Testing and Materials, Philadelphia, PA.

Crowell, H. 1979. Chemical control of terrestrial slugs and snails. Station Bulletin 628. Agricultural Experiment Station, Oregon State University. Corvallis, OR.

US EPA. 1985. Hazard evaluation division, Standard evaluation procedure. Acute toxicity test for freshwater invertebrates. 540/9-85/005. Office of Pesticide Programs. Washington, D.C.

Technique Name: Use of Small Mammals to Assess Exposure and Effects

Technique Type: Indicator Species
Matrix Type: Mammals
Ecosystem Level: Individual
Test Location: Field, Laboratory

Description:

Many species of small mammals have used to assess contaminant exposure potential or to assess contaminant effects. Three of the more commonly used small mammals are species of the genus Microtus (voles), Peromyscus leucopus (white-footed mouse), and Sigmodon hispidus (hispid cotton rat). Parameters that may be evaluated include the effects of exposure on reproductive effort, behavior, physiological parameters, immune system functioning, and DNA alterations. The mammals listed above have ranges that cover considerable porttions of the US and North America. Some species develop intricate burrow systems. The behavioral, morphological, and physiological characteristics of these organsisms have been extensively studied.

Logistical Considerations:

Sample Collection:

Training: MINIMAL

Time: MINIMAL - EXTENSIVE depending on the sample size required and study design.

Equipment: All the species listed can be easily captured using live traps or snap traps.

Sample Analysis:

Training: Not applicable. Depends on the analysis to be performed.

Time: Not applicable. Depends on the analysis to be performed.

Equipment: Not applicable. Depends on the analysis to be performed.

Critique/Comments:

The ubiquitous nature of rodents, their foraging habits, prolific reproductive potential, adaptability to laboratory setting, and the extensive database available in the literature make rodents excellent indicator species to model contaminant effects on small mammal communities and to assess trophic transfer of contaminants.

Key References:

Elangbam, C.S., C.W. Qualls, R.L. Lochmiller and J. Novak. 1989. Development of the cotton rat (Sigmodon hispidus) as a biomonitor of environmental contamination with emphasis on hepatic cytochrome P-450 induction and population characteristics. Bull. Environ. Contam. Toxicol. 42:482-488.

Health, V., D.J. Schaeffer, T.R. Seastedt, D.J. Gibson, D.C. Hartnett, B.A.D. Hetrick, S.W. James, D.W. Kaufman, A.P. Schwab, E.E. Herricks and E.W. Novak. 1990. Field bioassessments for selecting test systems to evaluate military training lands in tallgrass prairie ecosystem. Environ. Manage. 14:81-94.

McBee, K., J.W. Bickham, K.W. Brown and K.C. Donnelly. 1987. Chromosomal aberrations in native small mammals (Peromyscus leucopus and Sigmodon hispidus) at a petrochemical waste disposal site: I. Standard karyology. Arch. Environ. Contam. Toxicol. 16:681-688.

McBee, K. and J.W. Bickham. 1989. Mammals as bioindicators of environmental toxicity, In H.H. Genoways, ed., Current Mammalogy. Plenum Press, New York, pp. 37-88.

Thompson, R.A., G.D. Schroder and T.H. Connor. 1988. Chromosomal aberrations in the cotton rat, Sigmodon hispidus, exposed to hazardous waste. Environ. Molec. Mutagen. 11:359-367.

Technique Name: Use of Avian Species to Assess Exposure and Effects

Technique Type: Indicator Species
Matrix Type: Avian Species
Ecosystem Level: Individual
Test Location: Field, Laboratory

Description:

Many bird species have been used in ecological risk assessements to assess exposure potential for other species or to assess possible contaminant effects.

The great blue heron (Ardea herodius) can be used to evaluate effects of exposure to contaminants on wetland bird populations. Parameters that may be evaluated include the effects of exposure on reproductive effort, behavior, physiological parameters, immune system functioning, DNA alterations, and contamination of food items. The genus is ubiquitous throughout wetlands of North America. The diet of the great blue heron--composed of frogs, fish, and crustaceans--places it high in the food chain and increases its utility as a sentinel species for wetland birds.

The European starling (Sturnus vulgaris) can be used as a biomonitor for the effects environmental contaminants. Effects of toxicants can be evaluated on such variables as reproductive effort, growth and development, gross pathological abnormalities such as tumors and lesions, physiological impairments, and survival of adults and nestlings. Food items fed to nestlings can be examined to determine toxicant exposure through the diet. The species is particularly suited for field evaluations as the species readily utilizes artificial nesting boxes, allowing populations to be established in areas of known or suspected contamination, and is tolerant or repeated visitation to boxes by field personnel. The species is ubiquitous across North America.

Logistical Considerations:

Sample Collection:

Training: MINIMAL

Time: MINIMAL - EXTENSIVE depending on the sample size required and study design.

Equipment: The equipment required differs greatly among species. Many studies involve use radiotelemetry equipment.

Sample Analysis:

Training: Not applicable. Depends on the analysis to be performed.

Time: Not applicable. Depends on the analysis to be performed

Equipment: Not applicable. Depends on the analysis to be performed.

Critique/Comments:
Searching areas for natural nests is time-consuming, and needs to be repeated over several weeks, especially if data on renesting attempts and second clutches is needed. Nests of some species may also be relatively inaccessible, requiring the use of mirror-poles or ladders to examine the nests. Establishing nest boxes may be labor-intensive, as are platforms for raptors, although to a lesser extent as raptors are rarely abundant on study sites. Once avian nests have been identified or boxes and platforms established, an immense amount of data can thereafter be collected with relative ease. Through use of the artificial nesting structures, populations can be established on areas of known or suspected contamination and data collected to address various risk assessment objectives with minimal time and equipment.

Key References:

Grue, C.E. and C.C. Hunter. 1984. Brain cholinesterase activity in fledgling starlings: implications for monitoring exposure of songbirds to ChE inhibitors. Bull. Environ. Contam. Toxicol. 32:282-289.

Grue, C.E. and L.P. Franson. 1986. Use of captive starlings to determine effects of environmental contaminants on passerine reproduction: pen characteristics and nestling food requirements. Bull. Environ. Contam. Toxicol. 37:655-663.

Kendall, R.J., L.W. Brewer, T.E. Lacher, B.T. Marden and M.L. Whitten. 1989. The use of starling nest boxes for field reproductive studies: provisional guidance document and support documents. EPA/600/8-89/056. U.S. Environmental Protection Agency, Washington ,D.C.

Kessel, B. 1957. A study of the breeding biology of the European starling (Sturnus vulgaris) in North America. Am. Mid. Nat. 58(2):257-331.

Lower, W.R. and R.J. Kendall. 1990. Sentinel species and sentinel bioassays, In J.F. McCarthy and L.R. Shugart, eds., Biomarkers of Environmental Contamination. Lewis Publishers, Boca Raton, FL, pp. 168-179.

Technique Name: Avian Eggshell Thinning

Technique Type: Physiological
Matrix Type: Biological Tissue
Ecosystem Level: Individual
Test Location: Field

Description:

The thinning of avian eggshells has been observed as a response to various stressors including DDT metabolites, dieldrin, chlordecone, lindane, polychlorinated biphenyls, mercury, and aluminum. In the past, eggshell thickness has been assessed using a thickness index. Recently, a more sensitive and highly quantifiable technique has been developed in which the breaking strength of the egg is assessed. Eggshell thinning is a widely used tool in field assessments.

Logistical Considerations:

Sample Collection:

Training: MINIMAL

Time: MINIMAL - MODERATE

Equipment: No special equipment is required.

Sample Analysis:

Training: MINIMAL

Time: MINIMAL for individual samples.

Equipment: Typical laboratory equipment is required.

Critique/Comments:

Key References:

Bennett, J.K., R.K. Ringer, R.S. Bennett, B.A. Williams and P.E. Humphrey. 1988. Comparison of breaking strength and shell thickness as evaluators of eggshell quality. Environ. Toxicol. Chem. 7:351-357.

Carlisle, J.C., D.W. Lamb and P.A. Toll. 1986. Breaking strength: an alternative indicator of toxic effects on avian eggshell quality. Environ. Toxicol. Chem. 5:887-889.
Ratcliffe, D.A. 1967. Decrease in eggshell weight in certain birds of prey. Nature (London) 215:208-210.

Technique Name: Fish Survey

Technique Type: Survey of biota
Matrix Type: Freshwater
Ecosystem Level: Community/Individual
Test Location: Field

Description:

Fish are sampled using electrofishing techniques and/or various types of nets, and the number of each species in the samples is determined. Typical analyses of the data include relative abundance, species richness, and size structure. Population estimates may be determined if repeated samples are taken. Fish communities can be assessed using the Index of Biological Integrity, which was developed specifically to determine the effects of decreased habitat quality. Fish samples can also be taken for residue analysis for contaminants that bioaccumulate. Residues can be compared to limits for consumption set by the Food and Drug Administration. Other methods of contaminant effects include percentage of tumors, vertebral anomalies, disease and parasites, and fin erosion.

Logistical Considerations:

Sample Collection:

Training: MINIMAL

Time: MINIMAL

Equipment: An electrofishing unit is required if that technique is to be employed.

Sample Analysis:

Training: MODERATE

Time: MODERATE

Equipment: MINIMAL

Critique/Comments:

Information from residue analysis should be interpreted with caution, as many contaminants to which fish have been exposed will not show in the analysis due to degradation of the compound. Furthermore, it is difficult to relate body burdens of a

contaminant to potential biological effects. Findings of physical abnormalities should also be interpreted cautiously due to mobility of fish, statistical errors in inferences, differential species sensitivity, and subjectivity in observations.

Key References:

Baumann, P.C., W.K. Smith and W.K. Parland. 1987. Tumor frequencies and contaminant concentration of brown bullheads from an industrialized river and a recreational lake. Trans. Am. Fish. Soc. 116:251-253.

Bengtsson, B.E. 1975. Vertebral damage in fish induced by pollutants, In J.H. Kowman, J.J. Strik, eds., Sublethal Effects of Toxic Chemicals on Aquatic Animals. Elsevier Scientific, Amsterdam.

Karr, J.R., K.D. Fausch, P.L. Angermeier, P.R. Yant and I.J. Schlosser. 1986. Assessing biological integrity in running waters: A method and its rationale. Illinois Natural History Survey Special Publication No. 5, Illinois Natural History Survey, Champaign, IL.

Overstreet, R.M. and H.K. Howse. 1977. Some parasites and diseases of estuarine fishes in polluted habitats of the Mississippi. Ann. N.Y. Acad. Sci. 298:427-462.

Sherwood, M.J. and A.J. Mearns. 1977. Environmental significance of fin erosion in polluted habitats of the Mississippi. Ann. N.Y. Acad. Sci. 298:427-462.

Technique Name: Plankton Survey

Technique Type: Survey of flora and fauna
Matrix Type: Freshwater
Ecosystem Level: Community
Test Location: Lab

Description:

Samples of the plankton community are taken from the water column at a different depths using one or several of a variety of techniques. Plankton samples are then preserved for taxonomic identification. Species richness, relative abundance, and community indices can be determined from the taxonomic data.

Logistical Considerations:

Sample Collection:

Training: MINIMAL

Time: MINIMAL

Equipment: MINIMAL

Sample Analysis:

Training: MODERATE

Time: MODERATE

Equipment: A dissecting microscope may be needed for taxonomic evaluation.

Critique/Comments:

The choice of sampling technique, sample size, and sample numbers will depend on the characteristics of the habitat.

Key References:

American Society for Testing and Materials (ASTM). 1987. Standard practice for sampling phytoplankton with water-sampling bottles, In Annual Book of ASTM Standards: Water and Environmental Technology, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA, pp. 53-54.

American Society for Testing and Materials (ASTM). 1987. Standard

practice for sampling phytoplankton with pumps, In Annual Book of ASTM Standards: Water and Environmental Technology, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA, pp. 45-46.

American Society for Testing and Materials (ASTM). 1987. Standard practice for sampling phytoplankton with conical tow nets, In Annual Book of ASTM Standards: Water and Environmental Technology, Vol. 11.04. American Society for Testing and Materials, Philadelphia, PA, pp. 42-44.

DeBernardi, R. 1984. Methods for the estimation of zooplankton abundance, In J.A. Downing and F.H. Rigler, eds., A Manual on Methods for the Assessment of Secondary Productivity of Fresh Waters, IBP Handbook 17. Blackwell Scientific Publications, Oxford, England, pp 59-86.

Technique Name: Periphyton Survey

Technique Type: Survey of microflora
Matrix Type: Freshwater
Ecosystem Level: Community
Test Location: Lab

Description:

Changes in lotic systems resulting from contaminants can be assessed by surveys of the periphyton community. A sample of the periphyton community is obtained from natural substrate or artificial substrate implanted specifically for the purpose of colonization by periphyton. Samples are analyzed for taxonomic composition such as cell number, species richness, and relative abundance. Community indices such as diversity and community similarity and other productivity-related indices can also be determined.

Logistical Considerations:

Sample Collection:

Training: MINIMAL

Time: MINIMAL

Equipment: MINIMAL to MODERATE

Sample Analysis:

Training: MODERATE

Time: MINIMAL

Equipment: A microscope is needed for identification.

Critique/Comments:

Periphyton surveys should be supported by additional physical and chemical information, which sometimes influences periphyton production and dynamics.
Enough cells must be counted to ensure that rare cells are counted.

Key References:

American Public Health Association (APHA). 1985. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, DC.

Crossey, M.J. and T.W. La Point. 1988. A comparison of periphyton community structural and functional responses to heavy metals. Hydrobiologia 162:109-121.

Stevenson, R.J. and R.L. Lowe. 1986. Sampling and interpretation of algal patterns for water quality assessments, In B.G. Isom, ed., Rationale for Sampling and Interpretation of Ecological Data. ASTM STP 894. American Society for Testing and Materials. Philadelphia, PA. pp 118-149.

Technique Name: Analysis of Benthic Macroinvertebrate Populations

Technique Type: Survey of benthic biota
Matrix Type: Benthic habitat
Ecosystem Level: Community
Test Location: Field

Description:

Benthic macroinvertebrates are the most common fauna used in ecological assessments of contaminants. Typically, macroinvertebrates are sampled from the natural benthic habitat using one or several of a variety of techniques. An alternative sampling technique is to place artificial substrate into the water and then to collect the substrate after a period of colonization (usually about 6 weeks). Various information can be gleaned from macroinvertebrate surveys including relative abundance; species richness; guild structure; and indices of diversity, evenness, and community similarity.

Logistical Considerations:

Sample Collection:

Training: MINIMAL

Time: MODERATE

Equipment: MINIMAL

Sample Analysis:

Training: MINIMAL

Time: MODERATE

Equipment: MINIMAL

Critique/Comments:

Numerous excellent references deal with the collection, identification, and analysis of benthic invertebrate populations. It is essential that the sampling technique chosen be adequately suited for the target taxa and habitat type. The amount of time and training required for sample analysis varies depending on the taxonomic resolution desired.

Key References:

American Public Health Association (APHA). 1985. Standard Methods for the Examination of Water and Wastewater. American Public Health Association. Washington, D.C.

Downing, J.A. 1984. Sampling the benthos of standing waters, In J.A. Downing and F.H. Rigler, eds., A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters, IBP Handbook 17. Blackwell Scientific Publications, Oxford, England, pp. 87-103.

Merritt, R.W. and K.W. Cummins, eds. 1984. An Introduction to the Aquatic Insects of North America. Kendall/Hunt Publ., Dubuque, IA.

Peckarsky, B.L. 1984. Sampling the stream benthos, In J.A. Downing and F.H. Rigler, eds., A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters, IBP Handbook 17. Blackwell Scientific Publications, Oxford, England, pp. 131-160.

Southwood, T.R.E. 1978. Ecological Methods. John Wiley and Sons, New York, NY.

Technique Name: Presence/Absence of Indicator Species

Technique Type: Survey of biota
Matrix Type: Aquatic
Ecosystem Level: Individual
Test Location: Field

Description:

The indicator species concept was based originally on the premise that an increase in anthropogenic organic matter provides the food energy required by "tolerant" species, while the numbers of "sensitive" species declines in response to increased competition, predation, or decreased dissolved oxygen. Under this concept, the presence of sensitive species at a contaminated site led to the conclusion that there was little impact on the aquatic community. However, this approach holds limited applicability to contaminants other than organic matter. Currently, the indicator species concept is utilized by comparing changes in taxa numbers between a control site and hazardous waste sites. Effects of the contaminants can be assessed by assuming that an adverse effect on the community will be reflected by a decline in the number of members in more sensitive taxa.

Logistical Considerations:

Sample Collection:

Training: MINIMAL

Time: MINIMAL

Equipment: An electroshocking unit is recommended for fish censusing.

Sample Analysis:

Training: MODERATE

Time: MODERATE

Equipment: MINIMAL

Critique/Comments:

The use of indicator species to assess the effects of toxicants can be a useful tool if care is taken to carefully limit its

application. Communities do not respond similarly to different toxicants, so it is necessary to carefully consider the toxicant, mode of exposure, and community at risk when designing the study.

Key References:

Karr, J.R., K.D. Fausch, P.L. Angermeier, P.R. Yant and I.J. Schlosser. 1986. Assessing biological integrity in running waters: A method and its rationale. Illinois Natural History Survey Special Publication No. 5, Illinois Natural History Survey, Champaign, IL. 28pp.

Plafkin, J.L., M.T. Barbour, K.K. Porter and S.K. Gross. 1988. Rapid bioassessment protocols for use in streams and rivers: Benthic macroinvertebrates and fish. Draft Report RT182A, from EA Engineering, Science and Technology Inc. to the U.S. Environmental Protection Agency, Monitoring, and Data Support Division, Washington, DC.

Technique Name: **Remote Sensing of Vegetation**

Technique Type: Remote Sensing
Matrix Type: Vascular Plant (terrestrial)
Ecosystem Level: Organismal, Community
Test Location: Field

Description:

Remote sensing may be used advantageously in a number of ways to assess vegetation of hazardous waste sites. Extensive efforts are underway in the U.S. National Aeronautics and Space Administration (NASA) and to a limited extent in EPA to characterize regional patterns in vegetation. Primary sources of radiometric data are the Landsat Multi Spectral Scanner (MSS), the Thematic Mapper (TM), and the French Systeme Probatoire d'Observation de la Terre (SPOT) data banks. Resolution is the major limitation of these satellite imaging systems. For improved resolution, the satellite images may be supplemented with fixed-wing aircraft (including ultralights) utilizing comparable sensing equipment. The flights may also employ infrared and conventional photography. Coordinated work at individual sites for verification ("ground truthing") or for additional resolution can be performed from "cherry picker" booms with field model sensors. These different levels of resolution provide the following opportunities: relatively unlimited accessibility; safe, non-intrusive assessment and monitoring; and the opportunity to assess large-scale seasonal and annual vegetational patterns. Radiometric data have been used effectively to map vegetational boundaries (detecting shifts in dominant canopy species within a given forest type), estimate net photosynthesis and net primary production, estimate foliar nitrogen content, detect drought stress, detect effects from pest epidemics such as gypsy moth, and assess forest decline due to air pollutants.

Logistical Considerations:

Sample Collection:

Training: MODERATE to EXTENSIVE.

Time: MINIMAL to EXTENSIVE depending on size of area.

Equipment: Very EXPENSIVE.

Sample Analysis:

Training: MODERATE to EXTENSIVE.

Time: MINIMAL to EXTENSIVE.

Equipment: A computer with digitizing board, or a radiometer is needed.

Critique/Comments:

Remote sensing and radiometry techniques can be useful in identifying areas of a hazardous waste site that have been negatively impacted by contaminants. However, verification or "ground truthing" (i.e., direct visual observation, chemical analysis of plant tissue and soils) must also be performed to insure that the impacted area is being affected by the contaminant(s) in question. The methods, whether satellite images, aerial photos or hand-held radiometers are used, are time consuming and expensive. Also, extensive training is often required to accurately collect and interpret the data. A cost-effect analysis should be performed before these techniques are implemented in a risk assessment program.

Key References:

Daughtry, C.S.T. and L.L. Biehl. 1985. Changes in Spectral Properties of Detached Birch Leaves. Remote Sensing of Environment 17:281-289.

Duinker, P. and S. Nilsson. 1988. Proceedings: Seminary on remote sensing of forest decline attributed to air pollution. International Institute for Applied Systems Analysis, Luxenburg, Austria.

Hardisky, M.A., M.F. Gross, and V. Klemas. 1986. Remote Sensing of coastal wetlands. BioScience 36:453-460.

Rock, B.N., J.E. Vogelmann, D.L. Williams, A.F. Vogelmann, and T. Hoshizaki. 1986. Remote detection of forest damage. BioScience 36:439-445.

Roller, N.E.G. and J.E. Colwell. 1986. Coarse-resolution satellite data for ecological surveys. BioScience 36:468-475.

Waring, R.H., J.D. Aber, J.M. Melillo, and B. Morre, III. 1986. Precursors of change in terrestrial ecosystems. BioScience 36:433-438.

Technique Name: **Digital Imaging Analysis of Vegetation**

Technique Type: Digital Imaging
Matrix Type: Vascular Plant
Ecosystem Level: Organismal, Community
Test Location: Field or Laboratory

Description:

Images of plant communities, individual plants, or individual leaves, acquired from satellite, aerial, or hand-held cameras or sensing equipment, are analyzed for relative light reflectance. After the image is captured, it is digitized using an array of gray levels (pixels). Damaged areas of plant communities, individual plants, or leaves can be distinguished from "healthy" areas based on pixel intensity. Varying degrees of damage within an image can be further delineated using a pseudocolor system that assigns a color to each level of intensity. Damage can be quantified by measuring total pixel intensity of an image or by determining the difference in surface area between "damaged" and "healthy" areas of the vegetative tissue. Sensitivity can be increased by using near-infrared film or filters or by using filters with wavelengths similar to chlorophyll (i.e., 680 nm or 730 nm).

Logistical Considerations:

Sample Collection:

Training: MINIMAL.

Time: MINIMAL.

Equipment: A digital imaging analysis system (DIAS), including video camera, digitizing board, computer, video monitor, and supplemental lighting is required.

Sample Analysis:

Training: MINIMAL.

Time: MINIMAL.

Equipment: A DIAS system is required.

Critique/Comments:

Digital imaging analysis can be a quick, accurate method for determining foliar injury in individual leaves, whole plants or small field plots. The method provides more accurate estimates of injured tissue surface area, and homogeneity among rating times and evaluators than visual injury estimations. Lighting, camera settings (i.e., F-stop, focus, contrast) and pixel intensity levels must be optimized prior to analyses. Set-up and calibration of the system is critical. By using selective filters, it may be possible to detect injury at the cellular level that may not be detected visually. Analysis can easily be incorporated into standard 14-day early seedling growth and vigor tests to detect subtle, chronic effects of contaminants. Imaging of areas acquired by satellite or aerial photography can determine differences in vegetation growth or canopy cover, but additional visual surveys, biomass measurements, or chemical analyses may be required to verify contaminant effects.

Key References:

Hader, D.P. 1988. Computer-assisted image analysis in biological sciences. Proc. Indian Acad. Sci. 98:227-249.

Stutte, C.A. and G.W. Stutte. 1988. An interactive image capture and analysis system (ICAS) for research and crop management, p. 151-159. In P. Mausel (ed.). Videography: First workshop. Amer. Soc. Photogram. and Remote Sens., Falls Church, VA.

Stutte, G.W. 1989. Quantification of net enzymatic activity in developing peach fruit using computer video image analysis. HortScience 23:113-115.

Stutte, G.W. 1990. Analysis of video images using an interactive image capture and analysis system. HortScience 25(6):695-697.

Stutte, G.W., R. Bors, and C.A. Stutte. 1990. Quantification of nutrient stress in horticultural crops using videography. Volume 16, Number 3. Journal of Imaging Technology 16:124-127.

Technique Name: **Plant Ecological Surveys**

Technique Type: Survey
Matrix Type: Terrestrial and Wetland Ecosystems
Ecosystem Level: Community
Test Location: Field

Description:

Plant communities are surveyed in the field using plot (grid), transect, and/or point-quarter sampling techniques. The plot technique involves dissecting an area into a grid system. Cells are selected randomly within each grid, plots are positioned within each cell through some unbiased "random process" (e.g., a random number of paces north and west of a designated point within each cell). Transect sampling involves establishment of a line following a compass bearing. Sampling occurs at pre-determined regular or random intervals along the line. In point-quarter sampling, a number of randomly-determined points are selected within a stand. Each point represents the center of four compass directions (N,S,E,W), that divide the sampling site into four quadrants. In each quadrant, the distance from the center point to the center of the nearest individual is measured. Endpoints include community structure (i.e., species identification, plant form, foliage density, frequency, biomass, coverage, community similarity, and ecological succession) species diversity within a community, community similarity, and ecological succession.

Logistical Considerations:

Sample Collection:

Training: MINIMAL to establish plots and collect data.

Time: MODERATE to EXTENSIVE (a few weeks to a few years) depending on the size of the area and amount and type of sampling required.

Equipment: MINIMAL.

Sample Analysis:

Training: MODERATE to EXTENSIVE to identify species and calculate ecological endpoints.

Time: MODERATE to EXTENSIVE.

Equipment: A computer is required for data compilation and statistical analyses.

Critique/Comments:

Implementation of these methods often requires considerable training and time consumption. These methods should only be used when a detailed analysis of the plant ecology is required. These methods should also be done in conjunction with short-term toxicity tests. Extreme caution must accompany any interpretation of synthetic indices, e.g., community structure and species diversity since natural selection and stress effect the diversity of a community in non-linear patterns. A good characterization of the soils should be made, as well as an analysis of other confounding factors (i.e., ambient air pollutants such as O_3 and SO_2) to delineate site effects from contaminant effects. Furthermore, diversity may increase or decrease at a hazardous waste site. Qualitative values of harm or benefit cannot be assigned to fluxes in diversity without careful ecological analysis of the underlying features affecting a given change.

Key References:

Bonham, C.D. 1989. Measurements for terrestrial vegetation. John Wiley & Sons. Inc. New York, NY. 338 pp.

Cox, G.W. 1985. Laboratory Manual of General Ecology. W.C. Brown, Dubuque, LA.

Green, R.H. 1979. Sampling design and statistical methods for environmental biologists. Wiley Interscience.

Greig-Smith, P. 1983. Quantitative Plant Ecology. Third Edition. University of California Press, Berkeley. 359 pp.

Meyers, W.L. and R.L. Shelton. 1980. Survey Methods for Ecosystem Management. John Wiley & Sons, New York, NY.

Technique Name: **Soil Fauna Microcosm**

Technique Type: Community and Trophic Level Analysis
Matrix Type: Soil Microfauna
Ecosystem Level: Community
Test Location: Laboratory

Description:

This technique evaluates the effects of contaminants on community structure of soil-borne nematodes and microarthropods using a soil microcosm. A field soil is collected, sieved, and mixed with contaminant solutions at various treatment levels. The treated soils are placed into plastic leach tubes and incubated at room temperature (18-21°C) for 7-14 days. Soil is then removed from each tube, gently mixed, and then subsampled for chemical concentration, percent moisture and soil nematodes and arthropods. To extract nematodes, soil fractions are placed on Baermann funnels for 48 hours at room temperature. Nematodes are counted live, identified taxonomically, and sorted into fungivore, bacteriovore, herbivore, and omnivore-predator trophic groups. Hatchlings are also counted. For extraction of microarthropods, soil fractions are extracted into 95% ethanol from Merchant-Crossley high-gradient tullgren extractors for 7 days at 42°C. Microarthropods are sorted into acarine suborders Prostigmata, Mesostigmata, and Oribatida, the insectan order Collembola, and "other" miscellaneous arthropods. Community structure and trophic level analysis is performed and differences among treatment levels is determined.

Logistical Considerations:

Sample Collection:

Training: MINIMAL

Time: MINIMAL

Equipment: MINIMAL

Sample Analysis:

Training: MINIMAL to recognize and count microfauna.
MODERATE to conduct community and trophic level analysis.

Time: MODERATE.

Equipment: A 140-power dissecting microscope is required to identify and count microfauna.

Critique/Comments:

The soil fauna microcosm is a simple inexpensive assay to measure contaminant effects on soil invertebrates using trophic structure and community analysis. This method has an advantage over single-species tests because it can be used for site-specific ecotoxicological studies of pollutants on communities of native species occupying many trophic levels in the soil system. Therefore, higher resolution of ecotoxicological effects in complex soil systems can be obtained by this approach, rather than by single-species based methods. This is a new method that has been tested with only a few chemicals and, therefore, no database has been established. Furthermore, considerable training is needed to conduct community and trophic level analysis and only a few laboratories have the expertise to do these studies. However, the test has a lot of potential for use in ecological risk assessments.

Key References:

Anderson, J.M. 1988. Spatiotemporal effects of invertebrates on soil processes. Biol. Fertil. Soils 6:216-227.

Moore, J.C. and P.C. De Ruiter. 1990. Temporal and spatial heterogeneity of trophic interactions within belowgrown food webs. In: Crossley, D.A. Jr. (ed.), Modern Techniques in Soil Ecology. Elsevier, Amsterdam. pp 371-398.

Parmelee, R.W. and D.G. Alston. 1986. Nematode trophic structure in conventional and no-tillage agroecosystems. J. Nematol. 18:403-407.

Parmelee, R.W., R.S. Wentsel, C.T. Phillips, M. Simini, and R.T. Checkai. 1993. A soil microcosm for testing the effects of chemical pollutants on soil fauna communities and trophic structure. Environ. Tox. Chem. (In press).

Petersen, H. and M. Luxton. 1982. A comparative analysis of soil fauna populations and their role in decomposition processes. Oikos 39:287-388.

Technique Name: **Bacterial Biomass in Soils**

Technique Type: Soil Survey
Matrix Type: Soil Biota
Ecosystem Level: Kingdom, Community
Test Location: Field, Laboratory

Description:

Tests include direct estimates of total and active bacterial numbers, and community composition. Estimates of active bacteria in a sample involve extraction by shaking in buffer solution and staining with a solution of fluorescein diacetate (FDA). Number and diameter of all fluorescent bacteria are measured using epi-fluorescent microscopy at 1000X or greater total magnification. To estimate total bacterial numbers, each sample is diluted and stained with fluorescein isothiocyanate, filtered, de-stained, and counted using epi-fluorescent microscopy. Bacterial community composition assays test the effect of contaminant(s) on the number of sensitive species (if tagged with a immuno-fluorescent stain) and on bacterial ecosystems. Dilutions of soil are spread on a variety of different agar media in a variety of different abiotic conditions. The colonies of bacteria which appear are then isolated and identified. Bacterial identity is usually determined by ability to grow and catabolize specific test nutrients (e.g., various sugars, carbohydrates) or to produce specific enzymes (dehydrogenase, oxidase, etc.).

Logistical Considerations:

Sample Collection:

Training: MINIMAL to grow, extract, and isolate bacteria.

Time: MINIMAL (< 7 days).

Equipment: MINIMAL for estimates of active and total bacterial numbers. An incubator is required forcommunity composition tests.

Sample Analysis:

Training: MINIMAL.

Time: MINIMAL.

Equipment: A light microscope equipped to view

fluorescent bacteria is required.

Critique/Comments:

Several research publications have suggested that changes in bacterial activity and biomass indicate possible changes in decomposition rates, soil fertility, and general ecosystem function. However, total bacterial biomass is relatively constant over all ecosystem and soil types, suggesting that this metric is less sensitive to disturbance with respect to other soil foodweb determinations. Few studies have measured active bacterial numbers using FDA. In combination with information on active and total fungal biomass, protozoan numbers and community structure and nematode numbers and community structure, nutrient cycling, energy flow, foodweb structure and diversity can be estimated. Regulatory standards exist for numbers and types of bacteria in water and wastewater. These measurements are performed by plating on a general medium (total aerobic bacteria) and media specific to coliforms. In soil, there is no known group of bacteria with a comparable indicative function such as coliforms have in water. Much more work is needed in soils to relate species presence, function and total numbers to a regulatory role.

Key References:

Babiuk, L.A. and E.A. Paul. 1970. The use of fluorescein isothiocyantate in the determination of the bacterial biomass of a grassland soil. Can. J. Microbiol. 16:57-62.

Coleman, D.C. 1985. Through a ped darkly: an ecological assessment of root-soil-microbial-faunal interactions. In A. H. Fitter, D. Atkinson, D.J. Read, and M.B. Usher (eds.), Ecological Interactions in Soil. Blackwell Scientific Publications, Cambridge, U.K. pp. 1-21.

Domsch, K.H. and G. Jangnow. 1990. Soil bacteria. pg. 1-48 In Dindal, D. 1990. Soil Biology Guide. John Wiley and Sons. 1349 pp.

Nannipieri, P., S. Grego, and B. Ceccanti. 1990. Ecological significance of the biological activity in soil. Soil Biochemistry 6:293-355.

Technique Name: **Fungal Biomass in Soils**

Technique Type: Soil Bioassay
Matrix Type: Soil Fungi
Ecosystem Level: Kingdom, Community
Test Location: Laboratory

Description:

Three methods are primarily used to determine fungal biomass in soils: 1) Homogenization of substrate, dilution with agar solution, gelling of agar to make a thin film, and phase-contrast microscopic counting of hyphae; 2) Homogenization of substrate, staining with fluorescing dye, membrane filtration, and microscopic counting of fluorescent hyphae; 3) chemical clearing of litter substrate, application of a stain, and microscopic counting of stained hyphae within intact substrate. Various combinations of, and modifications to these techniques have been implemented. Active and total fungi can be determined. Lengths and diameters of fungal hyphae are determined by using a pre-calibrated grid. Total hyphae biomass is then calculated per sample. Community composition may also be determined by spreading soil dilutions on a variety of agar media, isolating colonies of fungi that appear, and identifying these fungi.

Logistical Considerations:

Sample Collection:

Training: MINIMAL to extract, stain, and culture fungi.

Time: MINIMAL.

Equipment: MINIMAL.

Sample Analysis:

Training: MINIMAL for fungal biomass measurements. MODERATE to EXTENSIVE for identification of fungi.

Time: MINIMAL for biomass measurements, MODERATE to EXTENSIVE for fungal identification and community composition.

Equipment: A compound light microscope with phase-contrast or fluorescence capabilities is required.

Critique/Comments:

Any soil, sediment, litter or plant material can be tested using this method. Sensitive species can be added and assayed using this approach, as long as survival and growth requirements for the particular species is known for the material being tested. Determination of total and active fungal length and biomass indicates effects of toxics on fungal activity, function and total biomass. Reductions in toxicant-impacted soil as compared to controls, or expected levels given the soil type and organic matter level, indicate a negative effect on fungal activity and biomass. Development is needed to determine the quantitative levels which delineate impacts with different toxic chemicals in different soil types.

For many fungi so isolated, the requirements for it to fruit (sexual or asexual reproductive structures) are not known, and thus the fungus can not be identified. Additionally, the culture requirements for many soil fungi are not known and the percentage of the actual fungal community present in soil which grow on the agar media chosen cannot be determined.

Development of the database for a variety of soil types is currently underway and research is needed to determine the application of this test to Superfund and general regulatory settings. Soil biomass testing services are available at several land-grant institutions.

Key References:

Ingham, E.R. and D.A. Klein. 1984. Soil fungi: Relationships between hyphal activity and staining with fluorescein diacetate. Soil Biol. Biochem. 16:273-278.

Jones, P.C.T. and J.E. Mollison. 1948. A technique for the quantitative estimation of soil micro-organisms. J. General Microbiology 2:54-69.

Kendrick, W.B. and D. Parkinson. 1990. Soil Fungi. pg. 49-68. In Dindal, D. 1990. Soil Biology Guide. John Wiley and sons. 1349 pp.

Newell, S.Y. and R.E. Hicks. 1982. Direct-count estimates of fungal and bacterial biovolume in dead leaves of smooth cordgrass (Spartina alterniflora Loisel). Estuaries 5(4):246-260.

Olson, F.C.W. 1950. Quantitative estimates of filamentous algae. Trans. Am. Microscopy Soc. 69:272-279.

Paul, E.A. and R.L. Johnson. 1977. Microscopic counting and adenosine 5'-triphosphate measurement in determining microbial growth in soils. Appl. Environ. Microbiol. 34(3):263-269.

Technique Name: **Protozoan Numbers and Diversity**

Technique Type: Soil Survey
Matrix Type: Soil, Protozoans
Ecosystem Level: Community
Test Location: Field, Laboratory

Description:

Several methods are available to determine protozoan number and diversity in contaminated soil, soil eluates, sediment, litter, and ground water. The most commonly used methods are either direct observation following dilution or extraction, or separation techniques. These methods include: turbidity based on protozoan feeding rates; most probable number (MPN) using a compound microscope following dilution; direct observation of watered soil suspensions; staining and fixation, membrane filtration to enumerate testate amoebae; high resolution microscopy (phase contrast, differential interference, scanning electron microscopy); and density centrifugation followed by staining and fixation. End points are usually total numbers and community structure in contaminated vs. control substrates. Determination of numbers of each protozoan group, i.e., flagellates, testate amoebae, naked amoebae, and ciliates indicates effects of toxics on protozoan function and total biomass. Reductions in toxicant-impacted soil as compared to controls, or expected levels given the soil type and organic matter level, indicate a negative effect on protozoan biomass. Additional approaches for assessing protozoan numbers, especially of particular protozoan groups, are available from the American Society of Agronomy (Stout, et. al., 1992).

Logistical Considerations:

Sample Collection:

Training: MINIMAL to learn extraction, dilution, staining, and fixation techniques.

Time: MINIMAL for direct soil extractions, MINIMAL to MODERATE (a few weeks) for tests requiring incubation.

Equipment: MINIMAL for extraction and culture materials.

Sample Analysis:

Training: MODERATE to EXTENSIVE to identify and accurately count types of protozoa.

Time: MINIMAL to MODERATE for community analysis.

Equipment: Requires EXPENSIVE compound or electron microscope.

Critique/Comments:

As indicator organisms, protozoa are perhaps unsurpassed. Protozoa can be classified to genus and often to species based on morphology alone (Lee et. al. 1985). Protozoa occur in large numbers in natural ecosystems and "capture" of a representative picture of the entire community is not a problem, unlike mammals or birds. To improve the use of protozoa as indicators, however, a greater understanding of: 1) their response to disturbances beyond the normal seasonal cycle, 2) their habitat-specificity, and 3) their prey-preferences in specific habitats is needed. Efforts should be directed towards understanding changes in protozoan community composition in terrestrial systems. Soil sediment, litter or plant material can be tested using these methods. Sensitive species can be added and assayed, as long as survival and growth requirements for the particular species is known for the material being tested. Considerable training is required to identify genera and species, and to accurately assess community structure. Interferences in soil matrixes (i.e., poor visual resolution, adsorption of organisms to soil particles) decreases extraction efficiency and identification accuracy compared to aquatic samples. Furthermore, several useful keys for aquatic protozoa exist; however, a comprehensive taxonomic guide to soil protozoa is lacking. Development of the database for a variety of soil types is currently underway.

Key References:

Bamforth, S.S. 1991b. Enumeration of soil ciliate active forms and cysts by a direct count method. Agric. Ecosyst. Environ. 34:209-212.

Basel, R.M., E.R. Richter, and G.J. Banwart. 1983. Monitoring microbial numbers in food by density centrifugation. Appl. Environ. Microbiol. 45:1156-1159.

Darbyshire, J.F., R.E. Wheatley, M.P. Greaves, and R.H.E. Inkson. 1974. A rapid micromethod for estimating bacterial and protozoan populations in soil. Ecology 61:764-771.

Foissner, W. 1986. Soil protozoa: fundamental problems, ecological significance, adaptations, indicators of environmental quality, guide to the literature. Prog. Protist. 2:69-212.

Griffiths, B.S. and K. Ritz. 1988. A technique to extract, enumerate and measure protozoa from mineral soils. Soil

Biol. Biochem. 20:163-174.
Lee, J.J., S.H. Hutner, and E.D. Bovee. 1985. An Illustrated Guide to the Protozoa. Soc. of Protozoologists, Lawrence, Kansas. 629 pp.
Stout, J.D., S.S. Bamforth, and J.D. Lousier. 1992. Protozoa, p. 1103-1120. In A.L. Page et al. (ed.), Methods of Soil Analysis. Part 2. 3rd. ed. American Society of Agronomy, Madison, WI.

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APPENDIX C

TECHNICAL TEST METHODS UNDER DEVELOPMENT FOR ECOLOGICAL RISK ASSESSMENT

INTRODUCTION

This appendix includes a variety of assays or techniques presently used or under development by various experts. The methods are primarily biochemical, concern DNA adducts or metabolic processes. Although the techniques listed herein have been published and show great promise in assessing the effects of contaminants at the cellular and sub-cellular levels, they have not yet been adapted for use in ecological risk assessment. For these procedures to be useful in the regulatory arena of CERCLA, research is needed which integrates these methods to supplement and complement information on populations and community responses to hazardous wastes.

The fact that these techniques have not been applied in assessment of hazardous waste sites should not preclude their continued development nor hinder efforts to link sublethal responses to population responses (mortality, changes in reproductive status, emigration, etc.). Therefore, the authors feel these techniques should be included in this volume.

Technique Name: Adenosine Triphosphatase Activity

Technique Type: Enzyme Inhibition
Matrix Type: Biological
Ecosystem Level: Individual
Test Location: Laboratory

Description:

Adenosine triphosphatase is a Mg^{2+}-activated enzyme that uses the energy of ATP to transport Na^{+} and K^{+} across cellular membranes. Effects on enzyme activity may occur by a decrease in ATP concentrations or by a direct toxic effect on the enzyme. Adenosine triphosphatase is inhibited in vitro by a variety of organochlorine compounds and heavy metals. More field research needs to be conducted before this procedure can be utilized in field studies.

The majority of research with this enzyme has been conducted using aquatic organisms in controlled laboratory environments.

Key References:

Saunders, R.L., E.B. Henderson, P.R. Harmon, C.E. Johnston and K. Davidson. 1983. Physiological effects of low pH on the smolting process in Atlantic salmon, In R.H. Peterson and H.H.V. Hord, eds., Workshop on Acid Rain. p. 49.

Watson, T.A. and F.W.H. Beamish. 1980. Effect of zinc on branchial ATPase activity in vivo in rainbow trout, Salmo gairdneri (Richardson). J. Wildl. Dis. 13:263-270.

Technique Name: Adenylate Energy Charge

Technique Type: Physiological
Matrix Type: Biological Tissue
Ecosystem Level: Individual
Test Location: Laboratory

Description:

Adenylate energy charge (AEC) is a measure of the metabolic energy available to an organism from the adenylate pool, i.e. adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP). Concentrations of these molecules are highly regulated and have a strong influence on many metabolic processes. AEC is a direct calculation based on concentrations of ATP, ADP, and AMP. AEC has been used extensively to assess responses of a variety of organisms to various toxicants and conditions under laboratory conditions, but its potential value as a biomarker in environmental studies has not been determined.

Key References:

Ivanovici, A.M. and W.J. Wiebe. 1982. For working definition of "stress": a review and critique, In G.W. Barrett and R. Rosenberg, eds., Stress and Natural Ecosystems. John Wiley & Sons, Inc., New York, p.13-27.

Giesy, J.P., C.S., Duke, R.D. Bingham and G.W. Dickson. 1983. Phosphoadenylate concentrations and adenylate energy charge as an integrated biochemical measure of stress in invertebrates. The effects of cadmium on the freshwater clam Corbicula fluminea. Toxicol. Environ. Chem. 6:259-295.

Technique Name: Plant Enzyme Activity

Technique Type: Physiological
Matrix Type: Biological Tissue
Ecosystem Level: Individual
Test Location: Laboratory

Description:

Activities of various plant enzymes have been used to assess the effects of air pollutants. The activity of peroxidase has been used as a nonspecific marker of general metabolic shift while enzymes such as ribulose-1,5,-bisphosphate carboxylase/oxygenase have been used because of their importance in critical metabolic reactions. Other enzymes have been selected because of their high sensitivity to a particular contaminant. Plant enzymes that mediate detoxification of a contaminant or its products have the highest potential value as biomarkers of stress.

Key References:

Alscher, R., M. Franz and C.W. Jeske. 1987. Sulfur dioxide and chloroplast metabolism, In J.A. Saunders, L. Kosak-Channing and E.E. Conn, eds., Phytochemical Effects of Environmental Compounds. Plenum Publishing, New York, pp.1-28.

Byl, T.D., and S.J. Klaine. 1991. Peroxidase activity as an indicator of sublethal stress in the aquatic plant Hydrilla verticillata (Royle), In Gorsuch, J.W., W.R. Lower, M.A. Lewis, and W. Wang, eds., Plants for Toxicity Assessment: Second Volume. American Society for Testing and Materials, Philadelphia, PA. pp. 101-106.

Heath, R.L. 1988. Biochemical mechanisms of pollutant stress, In W.W. Heck, O.C. Taylor and D.T. Tingey, eds., Assessment of Crop Loss from Air Pollutants. Elsevier Applied Science, London, pp. 311-328.

Scholz, F., H.R. Gregorius and D. Rudin, eds., 1989. Genetic Effect of Air Pollutants in Forest Tree Populations. Springer-Verlag, Berlin, p. 201.

Technique Name: Scope for Growth

Technique Type: Physiological -- Energetics
Matrix Type: Whole Organism
Ecosystem Level: Individual
Test Location: Laboratory

Description:

Scope for growth (SFG) is an integrative approach to assessing the energy status of an organism. Energy available for growth and reproduction can be assessed by measuring energy absorbed from food, energy lost via respiration, and energy lost via excretion. This test requires that organisms be taken from the field and transported to a laboratory for measurements. SFG is a highly developed method that has been rigorously tested under field conditions. Research has shown that SFG is a good indicator of general ecosystem health.

Key References:

Bayne, B.L., D.A. Brown, K. Burns, D.R. Dixon, A. Ivanovici, D.R. Livingstone, D.M. Lowe, M.N. Moore, A.R.D. Stebbing and J. Widdows. 1985. The effects of stress and pollution on marine animals. Praeger Publishers, New York, p. 384.

Warren, G.E. and G.E. Davis. 1967. Laboratory studies on the feeding, bioenergetics and growth of fish, In S.K. Gerhuy, ed., The Biological Basis of Freshwater Fish Production. Blackwell Scientific Publications, Oxford, England, pp. 175-214.

Technique Name: Protein Synthesis

Technique Type: Physiological
Matrix Type: Biological Tissue
Ecosystem Level: Individual
Test Location: Laboratory

Description:

By measuring protein synthesis rate in certain tissues, the growth rate of an organism can be assessed. Protein synthesis is generally considered to be a nonspecific biomarker. Synthesis rate is assessed by exposing the organism to radiolabeled amino acids that will be incorporated in de novo synthesized proteins. By examining various tissues for radiolabeled amino acid residues, rates of protein synthesis can be assessed. This approach has been validated in field studies. Protein synthesis is generally considered to be a nonspecific biomarker.

Key References:

Aldeman, I.R. 1987. Uptake of radioactive amino acids as indices of current growth rate of fish: a review, In R.Summerfelt and G. Hall, eds., Age and Growth of Fish. Iowa State University Press, Ames, IA, pp. 65-80.

Lied, E. and G. Rosenlund. 1984. The influence of the ratio of protein energy to total energy in the feed on the activity of protein synthesis in vitro, the level of ribosomal RNA and RNA-DNA ration in white trunk muscle of Atlantic Cod (Gadus moshua). Comp. Bioch. Physiol. 77A:489-494.

Viarengo, A., M. Pertica, G. Mancinelli, R. Capelli and M. Orunesu. 1980. Effects of copper on the uptake of amino acids, on protein synthesis and on ATP content in different tissues of Mytilus galloprovincialis L. Mar. Environ. Res. 4:145-152.

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Technique Name: Oncogene Activation

Technique Type: DNA Modification
Matrix Type: Biological Tissue
Ecosystem Level: Individual
Test Location: Laboratory

Description:

Oncogene activation analysis can be used as a biomarker of specific DNA mutations associated with the formation of cancerous tumors. Oncogenes are specific genetic sequences that may be activated when a chemical carcinogen directly alters the base-pair sequence of the gene. Altered protein function, resulting from translation of the mutation, inhibits the ability of a cell to properly regulate growth and can lead to tumor formation. Several families of oncogenes have been described. Of those, the c-ras family is detected most often in animal tumors. Genetic mutations at other loci may serve to inactivate certain tumor suppressors. Several techniques have been used to detect genetic mutations at specific sites of concern within amplified DNA strands. Methods include restriction analysis, oligotide hybridization, direct DNA sequencing, RNase mapping, gel retardation, plaque screening assay, liquid hybrid selection, and nonradioactive restriction fragment length polymorphism. The time and costs associated with detection of oncogene activation varies with the procedure. Studies of oncogene activation have been conducted using environmental fish species.

Key References:

McMahon, G., L.J. Huber, M.J. Moore, J.J. Stegeman and G.N. Wogan. 1990. Mutations in c-Ki-ras oncogenes in diseased livers of winter flounder from Boston Harbor. Proc. Natl. Acad. Sci. U.S.A. 87:841-845.

Wirgin, I.I., D. Currie, C. Gorunwald and S.Y. Garte. 1989. Molecular mechanisms of carcinogenesis in a natural population of Hudson River fish. Proc. AACR Mtg. 30:194.

Technique Name: Genetic Mutation Rates

Technique Type: DNA Modification
Matrix Type: Biological Tissue
Ecosystem Level: Individual
Test Location: Laboratory

Description:

Certain highly conserved genes exhibit very little variation in base-pair sequences even between species in distantly related phyla. DNA sampled from species environmentally exposed to contaminants can be amplified. By analyzing these samples for variant base sequences, inferences can be drawn concerning the mutagenic effect of contaminants. Various procedures can be used to analyze samples.

Techniques that can be used to analyze samples are costly. Many laboratories are assessing background levels of genetic variation within and among natural populations of many species. This procedure has the advantage of detecting genetic mutations that have occurred through generations of organisms exposed to low levels of a mutagen.
Extensive research needs to be conducted using environmental species before the value of this technique as a biomarker can be assessed.

Key References:

Appels, R., and R. L. Honeycutt. 1986. rDNA: evolution of a billion years, In S.K. Dutta, ed., DNA Systematics. CRC Press, Boca Raton, FL, pp 81-135.

Moritz, C., T.E. Dowling and W.M. Brown. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Ann. Rev. Ecol. Syst. 18:269-292.